Disclosed herein are methods and compositions comprising anti-NRP1 antibodies for use in cancer therapy.

Study Design

Eligibility Criteria

- ECOG 0-2
- Advanced solid tumors
- Adequate hematologic and organ function
- No active CNS metastases
- No condition causing bevacizumab ineligibility

Dose Limiting Toxicities

- Grade > 3 non-hematologic or non-hepatic toxicity
- Key exceptions: HTN, proteinuria, acute infusion reaction

- Grade > 3 hepatic transaminases or alkaline phosphatase
- Select bevacizumab-related Grade > 1 or 2 AEs
  (e.g. pulmonary or CNS hemorrhage, ATE, GI perforation, RPLS)

**FIG. 1**

Abstract: Disclosed herein are methods and compositions comprising anti-NRP1 antibodies for use in cancer therapy.
with sequence listing part of description (Rule 5.2(a))
USE OF NEUROPILIN-1 ANTAGONISTS FOR THE TREATMENT OF CANCER

Related Applications
This application is a non-provisional application filed under 37 CFR 1.53(b)(1), claiming priority under 35 USC 119(e) to provisional applications numbered 61/334,317 filed May 13, 2010; and provisional application number 61/349,426 filed May 28, 2010, the contents 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 neuropilin-1 (NRP1) antagonists for use in 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. 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.

Angiogenesis is an important cellular event in which vascular endothelial cells proliferate, prune, and reorganize to form new vessels from preexisting vascular network. There is compelling evidence that the development of a vascular supply is essential for normal and pathological proliferative processes. 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 PIGF. VEGF-A primarily binds to two high affinity receptor tyrosine kinases, VEGFR-1 (Flt-1) and VEGFR-2 (Flk-1/KDR), the latter being the major transmitter of vascular endothelial cell mitogenic signals of VEGF-A.

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 such as that associated with cancer.

Neuropilin-1 (NRPI) is a multi-functional receptor that contributes to the development of the nervous and vascular systems. NRPI was described as a receptor that binds the semaphorin 3A ligand, acting with plexin co-receptors to regulate axon guidance (He and Tessier-Lavigne, Cell (1997) 90:739-51). It was later shown that NRPI also binds members of the vascular endothelial growth factor (VEGF) ligand family to mediate vascular development (Soker et al, Cell (1998) 92:735-45; Kawasaki et al, Development (1999) 126:4895-902). In addition, several studies have proposed a role for NRPI in tumor biology by regulating vascular and/or tumor cell functions (Bielenberg et al, Exp Cell Res (2006) 312:584-93). Pan et al, J Biol Chem (2007) 282:24049-56 showed that a monoclonal antibody that binds to NRPI reduced VEGF-mediated endothelial cell migration in vitro. Blocking VEGF interaction with NRPI in vivo reduced angiogenesis and vascular remodeling. The anti-NRPI antibody slowed tumor growth as a single agent; it is proposed that this is due to anti-NRPI antibody-mediated reduction of vessel sprouting through a VEGF-dependent process. The anti-NRPI antibody enhanced the anti-angiogenic and anti-
tumor effects of VEGF blockade with an anti-VEGF antibody. The data suggest that by reducing vascular remodeling with anti-NRPI, vessels are likely to retain a more immature phenotype. As immature vessels are believed to be more VEGF-dependent, blood vessels in anti-NRPI-treated tumors may be rendered more susceptible to anti-VEGF therapy, thus resulting in combination efficacy in tumor models when combining both therapies (Pan et al., Cancer Cell (2007) 11:53-67).

Since cancer remains one of the most deadly diseases additional treatments 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 invention herein concerns the results obtained in clinical studies of an anti-NRPI antibody in human subjects diagnosed with cancer. The results indicate that, in some patients, administration of anti-NRPI antibody was associated with one or more adverse infusion reactions.

Accordingly, the invention provides a method of treating a patient diagnosed with cancer, comprising administering to the patient an effective amount of an anti-NRPI antibody, wherein prior to administration of the anti-NRPI antibody the patient is administered premedication to prevent or ameliorate infusion reactions associated with administration of the anti-NRPI antibody. The invention also provides a method of treating a patient diagnosed with cancer, comprising administering to the patient premedication to prevent or ameliorate infusion reactions associated with administration of an anti-NRPI antibody, and then administering to the patient the anti-NRPI antibody. In some embodiments, the method further comprises increasing infusion time of administering the anti-NRPI-antibody to the patient. For example, the infusion time is increased from about 90 minutes to about 120 minutes. In some embodiments, the method further comprises administering a chemotherapeutic agent to the patient. In some embodiments the method further comprises administering an anti-VEGF antibody to the patient.

In certain embodiments the premedication comprises administration of dexamethasone. In some embodiments the premedication comprises administration of one or more of the following compounds:, diphenhydramine, H2 receptor antagonist, HI receptor antagonist, corticosteroids (e.g. dexamethasone), non-steroidal anti-inflammatory drugs
(NSAIDs) and acetaminophen. The dexamethasone may be administered to the patient the same day as administration of the anti-NRPl antibody. In some methods of the invention the patient is additionally administered dexamethasone the day before administration of the anti-NRPl antibody. For example, the patient is administered 20 mg dexamethasone the day before administration of anti-NRPl antibody and is administered 10 mg dexamethasone, 50 mg of diphenhydramine, an H2 receptor antagonist and 650 mg of acetaminophen the same day as administration of the anti-NRPl antibody.

In each of the methods or compositions of the invention the anti-NRPl antibody may be substituted with a NRPl specific antagonist as described below. In certain embodiments of the methods or compositions of the invention, the anti-NRPl antibody can be a monoclonal antibody, a chimeric antibody, a fully human antibody, or a humanized antibody. The antibody, or antigen-binding fragment thereof, can also be an antibody that lacks an Fc portion, an F(ab')2, an Fab or an Fv structure. In any of the methods or compositions of the invention the anti-NRPl antibody can be MNRP1685A. Also contemplated are methods or compositions of the invention using an anti-NRPl antibody that competes with MNRP1685A for binding to NRPl or an anti-NRPl antibody that binds to the same epitope as MNRP1685A.

In certain embodiments the anti-NRPl antibody has a heavy chain variable region comprising the following amino acid sequence:

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EVQLVESGGG LVQPGGLRL SCAASGFTFS SYAMSWVRQA PGKLEWVSQ ISPAGGYTNY ADSVKGRFTI SADTSKNTAY LQMNSLRAED TAVYYCAREL PVCN\NMKVMD VWGQGTLVTVSS (SEQ ID NO: 1),
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and a light chain variable region comprising the following amino acid sequence:

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DIQMTQSPSS LSASVGRVT ITCRASQYFSSYLAWYQQKPGKAPKLLIYG ASSRASGVPS RFSGSGSGTD FTLTISSLQPEDFAYYCVQYLGSPTFGQGTKVEIKR (SEQ ID NO:2).
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In some embodiments the anti-NRPl antibody used in any of the methods or compositions of the invention competes with an anti-NRPl antibody having a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 1, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 2 for binding to NRPl or binds to the same epitope as the anti-NRPl antibody having such heavy and light chain variable region sequences.
In some embodiments, the anti-NRPI antibody comprises a CDRH1 comprising the following amino acid sequence: TFSSYAMS (SEQ ID NO:3), a CDRH2 comprising the following amino acid sequence: QISPAGGYTNVSADVKG (SEQ ID NO:4), a CDRH3 comprising the following amino acid sequence: GELPYYRMSKVMR (SEQ ID NO:5), a CDRL1 comprising the following amino acid sequence: RASQYFSSYLA (SEQ ID NO:6), a CDRL2 comprising the following amino acid sequence: ASSRASG (SEQ ID NO: 7), and a CDRL3 comprising the following amino acid sequence: QQYLGSVPT (SEQ ID NO:8). In some embodiments the anti-NRPI antibody used in any of the methods or compositions of the invention competes with the anti-NRPI antibody comprising a CDRH1 comprising the amino acid sequence of SEQ ID NO:3, a CDRH2 comprising the amino acid sequence of SEQ ID NO:4, a CDRH3 comprising the amino acid sequence of SEQ ID NO:5, a CDRL1 comprising the amino acid sequence of SEQ ID NO: 6, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 7, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 8 for binding to NRPI or binds to the same epitope as the anti-NRPI antibody comprising such CDR sequences.

The anti-VEGF antibody used in any of the methods or compositions of the invention can be a monoclonal antibody, a chimeric antibody, a fully human antibody, or a humanized antibody. The antibody, or antigen-binding fragment thereof, can also be an antibody that lacks an Fc portion, an F(ab')2, an Fab or an Fv structure. 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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EVQLVESGGGLVQPILSLRL SCAASGYFTFYNYCHRWRQA
PGKLEWVGWINTYTGEPY TADFKRRRTF SLTSDKSTAY LQMNSLRAED
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and a light chain variable region comprising the following amino acid sequence:

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DIQMTQSPSS LSASVGRVT ITCASQDIS NYLNWYQQKP GAKPVLITYF
TSSLHSGVPS RFSGSGGTG TDFTLITSSLQP EDFAFYYCQQ YSTVPWTFGQ
GTVKVEIKR (SEQ ID NO: 10).
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In some embodiments the anti-VEGF antibody comprises a CDRH1 comprising the following amino acid sequence: GYTFTNYGMN (SEQ ID NO: 11), a CDRH2 comprising the following amino acid sequence: WINTYTGEPYAADFKR (SEQ ID NO: 12), a CDRL3 comprising the following amino acid sequence: RFSGSGGTSQ TDFTLITSSLQP EDFAFYYCQQ YSTVPWTFGQ GTVKVEIKR (SEQ ID NO: 10).
comprising the following amino acid sequence: YPHYYGS SHWYFD V (SEQ ID NO: 13), a CDRL1 comprising the following amino acid sequence: SASQDISNYLN (SEQ ID NO: 14), a CDRL2 comprising the following amino acid sequence: FTSSLHS (SEQ ID NO: 15) and a CDRL3 comprising the amino acid sequence: QQYSTVPWT (SEQ ID NO: 16). 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 the patient in the methods of the invention is diagnosed with colorectal cancer, breast cancer, lung cancer, renal cancer, ovarian cancer or glioblastoma.

In other embodiments, treatment with the anti-NRPI antibody (with or without treatment with anti-VEGF antibody) 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 anti-NRPI antibody 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 anti-NRPI antibody. 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 certain embodiments the chemotherapy is
paclitaxel. In some aspects, the chemotherapeutic agent and the VEGF-specific antagonist are administered concurrently. In 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 anti-NRP1 antibody.

Also provided is a kit for treating a patient diagnosed with cancer, comprising a package, wherein the package comprises an anti-NRP1 antibody composition and instructions for using the anti-NRP1 antibody composition in cancer therapy, wherein the instructions recite that the patient should be premedicated to ameliorate infusion reactions associated with administration of the anti-NRP1 antibody.

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

Brief Description of the Drawings

Figures 1 and 2 show certain elements of the study design described in Example 1 below. Figure 3 summarizes patient characteristics for the trial described in Example 1. Figures 4 and 5 shows data on the pharmacokinetics of MNRP1685A. Figure 6 summarizes the adverse events observed in the trial described in Example 1. Figure 7 is a graph showing infusion related symptoms data from the trial described in Example 1. Figure 8 is a graph showing P1GF as a pharmacodynamic marker of systemic NRP1 pathway inhibition. Figure 9 is a graph showing how long patients were on the study described in Example 1.

Detailed Description

I. Definitions

"Neuropilin" or "NRP" refers collectively to neuropilin-1 (NRP1), neuropilin-2 (NRP2) and their isoforms and variants, as described in Rossignol et al. (2000) Genomics 70:211-222. Neuropilins are 120 to 130 kDa non-tyrosine kinase receptors. There are multiple NRP-1 and NRP-2 splice variants and soluble isoforms. The basic structure of neuropilins comprises five domains: three extracellular domains (ala2, blb2, and c), a transmembrane domain, and a cytoplasmic domain. The ala2 domain is homologous to complement components C1r and C1s (CUB), which generally contains four cysteine residues that form two disculfid bridges. The blb2 domain is homologous to coagulation factors V and
VIII. The central portion of the c domain is designated as MAM due to its homology to meprin, A5 and receptor tyrosine phosphotase µ proteins. The ala2 and blb2 domains are responsible for ligand binding, whereas the c domain is critical for homodimerization or heterodimerization. Gu et al. (2002) *J. Biol. Chem.* 277:18069-76; He and Tessier-Lavigne (1997) *Cell* 90:739-51.

"Neuropilin mediated biological activity" refers in general to physiological or pathological events in which neuropilin-1 and/or neuropilin-2 plays a substantial role. Non-limiting examples of such activities are axon guidance during embryonic nervous system development or neuron-regeneration, angiogenesis (including vascular modeling), tumorgenesis and tumor metastasis.

An "anti-NRPI antibody" is an antibody that binds to NRPI with sufficient affinity and specificity. The antibody selected will normally have a binding affinity for NRPI, for example, the antibody may bind hNRPI 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-NRPI antibody of the invention can be used as a therapeutic agent in targeting and interfering with diseases or conditions wherein the NRPI 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-NRPI antibody will usually not bind to other NRPs such as NRPI.

A "NRPI antagonist" refers to a molecule capable of neutralizing, blocking, inhibiting, abrogating, reducing or interfering with NRPI activities including its binding to one or more ligands such as VEGF. NRPI antagonists include anti-NRPI antibodies and antigen binding fragments thereof and small molecule inhibitors of NRPI.
The term "VEGF" or "VEGF-A" is used to refer to the 165-amino acid human vascular endothelial cell growth factor and related 121-, 145-, 189-, and 206- amino acid human vascular endothelial cell growth factors, as described by, e.g., Leung et al. Science, 246:1306 (1989), and Houck et al. Mol. Endocrin., 5:1806 (1991), together with the naturally occurring allelic and processed forms thereof. VEGF-A is part of a gene family including VEGF-B, VEGF-C, VEGF-D, VEGF-E, VEGF-F, and PIGF. VEGF-A primarily binds to two high affinity receptor tyrosine kinases, VEGFR-1 (Flt-1) and VEGFR-2 (Flk-1/KDR), the latter being the major transmitter of vascular endothelial cell mitogenic signals of VEGF-A. Additionally, neuropilin-1 has been identified as a receptor for heparin-binding VEGF-A isoforms, and may play a role in vascular development. The term "VEGF" or "VEGF-A" also refers to VEGFs from non-human species such as mouse, rat, or primate. Sometimes the VEGF from a specific species is indicated by terms such as hVEGF for human VEGF or mVEGF for murine VEGF. 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-1 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 P1GF, 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-
occuring truncated or secreted forms of the polypeptide (e.g., an extracellular domain
sequence), naturally-occurring variant forms (e.g., alternatively spliced forms) and naturally-
occuring 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(109) in the presence of a titration series of unlabeled VEGF, then capturing bound VEGF with an anti-Fab antibody-coated plate (Chen, et al, (1999) J. MolBiol. 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(109) are mixed with serial dilutions of a Fab of interest, e.g., Fab-12 (Presta et al, (1997) Cancer Res. 57:4593-4599). The Fab of interest is then incubated overnight; however, the incubation may continue for 65 hours to insure that equilibrium is reached. Thereafter, the mixtures are transferred to the capture plate for incubation at room temperature for one hour. The solution is then removed and the plate washed eight times with 0.1% Tween-20 in PBS. When the plates had dried, 150 ul/well of scintillant (MicroScint-20; Packard) is added, and the plates are counted on a Topcount gamma counter (Packard) for ten minutes. Concentrations of each Fab that give less than or equal to 20% of maximal binding are chosen for use in competitive binding assays. According to another embodiment the Kd or Kd value is measured by using surface plasmon resonance assays using a BIAcore™-2000 or a BIAcore™-3000 (BIAcore, Inc., Piscataway, NJ) at 25°C with
immobilized hVEGF (8-109) CM5 chips at -10 response units (RU). Briefly, carboxymethylated dextran biosensor chips (CM5, BIAcore Inc.) are activated with N-ethyl-N’-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) according to the supplier's instructions. Human VEGF is diluted with 10mM sodium acetate, pH 4.8, into 5ug/ml (~0.2uM) before injection at a flow rate of 5ul/minute to achieve approximately 10 response units (RU) of coupled protein. Following the injection of human VEGF, 1M ethanolamine is injected to block unreacted groups. For kinetics measurements, two-fold serial dilutions of Fab (0.78 nM to 500 nM) are injected in PBS with 0.05% Tween 20 (PBST) at 25°C at a flow rate of approximately 25ul/min. Association rates (k_on) and dissociation rates (k_off) are calculated using a simple one-to-one Langmuir binding model (BIAcore Evaluation Software version 3.2) by simultaneous fitting the association and dissociation sensorgram. The equilibrium dissociation constant (Kd) was calculated as the ratio k_off/k_on. See, e.g., Chen, Y., et al., (1999) J. Mol Biol 293:865-881. If the on-rate exceeds 10^6 M^-1 S^-1 by the surface plasmon resonance assay above, then the on-rate is can be determined by using a fluorescence quenching technique that measures the increase or decrease in fluorescence emission intensity (excitation = 295 nm; emission = 340 nm, 16 nm bandpass) 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 (ThermoScientific) 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 NRPl-specific antagonist antibody binds NRPl and inhibits the ability of NRPl to mediate one or more NRPl biological activities. 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 CHI domains; (ii) the Fab' fragment, which is a Fab fragment having one or more cysteine residues at the C-terminus of the CHI domain; (iii) the Fd fragment having VH and CHI domains; (iv) the Fd' fragment having VH and CHI domains and one or more cysteine residues at the C-terminus of the CHI 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* 242A 23-426 (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 93/1161; and Hollinger et al., *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993)); (xi) "linear antibodies" comprising a pair of tandem Fd segments (VH-CH1-VH-CH1) which, together with complementary light chain polypeptides, form a pair of antigen binding regions (Zapata et al. *Protein Eng.* 8(10):1057-1062 (1995); and US Patent No. 5,641,870).

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 (LI), 50-56 (L2) and 89-97 (L3) in the light chain variable domain and 31-35 (HI), 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 (LI), 50-52 (L2) and 91-96 (L3) in the light chain variable domain and 26-32 (HI), 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 CDRH1 includes amino acids H26-H35, the heavy chain FR1 residues are at positions 1-25 and the FR2 residues are at positions 36-49.

The "Fab" fragment contains a variable and constant domain of the light chain and a variable domain and the first constant domain (CHI) 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_H and V_L domains of antibody, wherein these domains are present in a single polypeptide chain. Generally the Fv polypeptide further comprises a polypeptide linker between the V_H and V_L domains, which enables the scFv to form the desired structure for antigen binding. For a review of scFv, see Pluckthun in *The Pharmacology of Monoclonal Antibodies*, Vol 113, Rosenberg and Moore eds. Springer-Verlag, New York, pp. 269-315 (1994).

The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy chain variable domain (V_H) connected to a light chain variable domain (V_L) in the same polypeptide chain (V_H and V_L). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are
forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully, for example, EP 404,097; WO 93/11161; 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_H-C_H 1-V_H-C_H 1) 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);

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 (l):86-95 (1991); and U.S. Pat. No. 5,750,373.

An "affinity matured" antibody is one with one or more alterations in one or more CDRs thereof which result an improvement in the affinity of the antibody for antigen, compared to a parent antibody which does not possess those alteration(s). Preferred affinity matured antibodies will have nanomolar or even picomolar affinities for the target antigen. Affinity matured antibodies are produced by procedures known in the art. Marks et al.

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 WO01/45746. 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-1 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).

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 NRP1-specific antagonist means that the NRP1-specific antagonist is administered in the absence of an additional anti-cancer therapy during treatment period.

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

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 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.

"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 "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.
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. At²¹¹, I¹³¹, I¹²⁵, Y⁹⁰, Re¹⁸⁶, Re¹⁸⁸, Sm¹⁵³, Bi²¹², P³² and radioactive isotopes of Lu), chemotherapeutic agents, and toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof.

A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include alkylating agents such as thiotepa and CYTOXAN® cyclophosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamlamines including altretamine, triethyleneemelamine, trietylennephosphoramid, triethylennethiophosphoramid and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); a camptothecin (including the synthetic analogue topotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, chlorophosphamide, 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 
edinye antibiotics (e. g., calicheamicin, especially calicheamicin gammall and 
dynemicin, including dynemicin A; bisphosphonates, such as clodronate; an esperamicin; as 
well as neocarzinostatin chromophore and related chromoprotein edinye antibiotic 
chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, 
cactinomycin, carabici, carminomycin, carzinophilin, chromomycinis, dactinomycin, 
daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, ADRIAMYCIN® doxorubicin 
(including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin 
and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such 
as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, 
puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, 
zorubicin; anti-metabolites such as methotrexate and 5-fluouracil (5-FU); folic 
acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; 
acitinoblastin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, 
potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, 
zorubicin; anti-metabolites such as methotrexate and 5-fluouracil (5-FU); folic 
analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine 
analogs such as ancitabine, azacitidine, 6-azauridine, carmob, 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; bestabucil; bisantrene; edatraxate; defofamine; 
demecoline; diaziquone; elfornithine; elliptinium acetate; an epothilone; etoglucid; gallium 
nitrate; hydroxyurea; lentinan; lomidainine; maytansinoids such as maytansine and 
ansamitocins; mitoguazone; mitoxantrone; mopardamol; nitraerine; pentostatin; phenamet; 
pirarubicin; losoxantrone; podophyllin acid; 2- ethylhydrazide; procarbazine; PSK® 
polysaccharide complex (JHS Natural Products, Eugene, OR); razoxane; rhizoxin; sizofiran; 
spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes 
-especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine;
dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside 
("Ara-C"); cyclophosphamide; thiotepa; taxoids, e.g., TAXOL® paclitaxel (Bristol- Myers
Squibb Oncology, Princeton, N.J.), ABRAXANE® Cremophor-free, albumin-engineered nanoparticle formulation of paclitaxel (American Pharmaceutical Partners, Schaumberg, Illinois), and TAXOTERE® doxetaxel (Rhone- Poulenc Rorer, Antony, France); chlorambucil; GEMZAR® gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin, oxaliplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; NAVELBINE® vinorelbine; novantrone; teniposide; edatrexate; daunomycin; aminopterin; xeloda; ibandronate; irinotecan (Camptosar, CPT-11) (including the treatment regimen of irinotecan with 5-FU and leucovorin); topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; capcitabine; 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, LY117018, 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, aminogluthethimide, 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 abherant cell proliferation, such as, for example, PKC-alpha, Raf 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-1alpha, 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 alkylating agents such as tamoxifen, prednisone, dacarbazine, mechlorethamine, cisplatin, methotrexate, 5-fluorouracil, and ara-C. Further information can be found in The Molecular Basis of Cancer, Mendelsohn and Israel, eds., Chapter 1, entitled "Cell cycle regulation, oncogenes, and antineoplastic drugs" by Murakami et al. (WB Saunders: Philadelphia, 1995), especially p. 13.
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.
A "disorder" is any condition that would benefit from treatment with the anti-NRPI 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.
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-NRPI Antibodies and Antagonists

(i) NRPI Antigen

The NRPI antigen to be used for production of antibodies may be derived from a mammalian species. Preferably the antigen is human NRPI (hNRPl). However, NRPs from other species such as murine NRPI (mNRPI) can also be used as the target antigen. The NRPI antigens from various mammalian species may be isolated from natural sources. In other embodiments, the antigen is produced recombinantly or made using other synthetic methods known in the art.

(ii) Anti-NRPI Antibodies

Anti-NRPI 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 NRPI and can reduce or inhibit one or more biological activities of NRPI. An anti-NRPI antibody will usually not bind to other NRPs such as NRP2.

In certain embodiments of the invention, the anti-NRPI antibodies include, but are not limited to, a fully human phage-derived anti-NRPI monoclonal antibodies as described in PCT Publication No. WO2007/056470 or U.S. Publication No. 20080213268 (U.S. Patent Application No. 12/107544), the content of these patent applications are expressly incorporated herein by reference. In one embodiment, the anti-NRPI antibody is YW107.4.87 as described in PCT Publication No. WO2007/056470 or U.S. Publication No. 20080213268 (U.S. Patent Application No. 12/107544), the content of these patent applications are expressly incorporated herein by reference.

In certain embodiments the anti-NRPI antibody has a heavy chain variable region comprising the following amino acid sequence:
EVQLVESGGG LVQPGGSLRL SCAASGFTFS SYAMSWVRQA PGKGEWVSVQ
ISPAGGYTNY ADSVKGRFTI SADTSKNTAY LQMNSLRAED TAVYYCARGEL
PYYRMSKVMD VWGQGTLVTVSS (SEQ ID NO: 1),

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

DIQMTQSPSS LSASVGDRVT ITCRASQYFSSYLA WYQQKPGKAPKLIVYG
ASSRASGVPS RFSGSGSGTD FTTLISSLQPEDFATYYCQQYLGSPPCTFKVEIKR
(SEQ ID NO: 2).

In some embodiments the anti-NRPI antibody used in any of the methods or compositions of
the invention competes with an anti-NRPl antibody having a heavy chain variable region
comprising the amino acid sequence of SEQ ID NO: 1, and a light chain variable region
comprising the amino acid sequence of SEQ ID NO: 2 for binding to NRPI or binds to the
same epitope as the anti-NRPI antibody having such heavy and light chain variable region
sequences.

In some embodiments, the anti-NRPI antibody comprises a CDRH1 comprising the
following amino acid sequence: TFSSYAMS (SEQ ID NO:3), a CDRH2 comprising the
following amino acid sequence: QISPAGGYTNYADSVKG (SEQ ID NO:4), a CDRH3
comprising the following amino acid sequence: GELPYRMSKVMDV (SEQ ID NO:5), a
CDRL1 comprising the following amino acid sequence: RASQYFSSYLA (SEQ ID NO:6), a
CDRL2 comprising the following amino acid sequence: ASSRASG (SEQ ID NO: 7), and a
CDRL3 comprising the following amino acid sequence: QQYLGSPPCT (SEQ ID NO:8). In
some embodiments the anti-NRPI antibody used in any of the methods or compositions of
the invention competes with the anti-NRPl antibody comprising a CDRH1 comprising the
amino acid sequence of SEQ ID NO:3, a CDRH2 comprising the amino acid sequence of
SEQ ID NO:4, a CDRH3 comprising the amino acid sequence of SEQ ID NO:5, a CDRL1
comprising the amino acid sequence of SEQ ID NO: 6, a CDRL2 comprising the amino acid
sequence of SEQ ID NO: 7, and a CDRL3 comprising the amino acid sequence of SEQ ID
NO: 8 for binding to NRPI or binds to the same epitope as the anti-NRPI antibody
comprising such CDR sequences.
III. Anti-VEGF Antibodies and Antagonists

(i) VEGF Antigen

The VEGF antigen to be used for production of antibodies may be, e.g., the VEGF<sub>65</sub> 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 <i>et al.</i> (1989) <i>Science</i>, 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 VEGF<sub>65</sub>. 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 <i>et al.</i> (1989) <i>Science, supra</i>.

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. (<i>See, e.g.,</i> Ferrara, <i>Laboratory Investigation</i> 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. VEGF<sub>121</sub> 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-Alam. 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 VEGF<sub>65</sub> homodimer.

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

(ii) 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 P1GF, 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, or competes for binding to VEGF, 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 IgGl framework regions and antigen-binding complementarity-determining regions from the murine anti-hVEGF monoclonal antibody A4.6.1 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 IgGl, 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 200501 12126; 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:

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EVQLVESGGG LVQPESGLRL SCAASGVTFT NYGMNWRVRQA PKGKLEWVWG
INTYTGEPY ADFKPRFTF SLDTSETAY LQMNLRAED TAVYYCAKYP
HYGGSHWYF DWGGQTLVT VSS (SEQ ID NO: 1)
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and a light chain variable region comprising the following amino acid sequence:

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DIQMTQSPSS LSASVGRVT ITCSASQDIS NYLNWYQQKP GAKPVLIPvF
TSSLHSGVPS RSFGSGSGTD FTLTSSLQP EDFATYYCQQ YSTYPWTFQG
GTVKEIKR (SEQ ID NO: 2).
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In some embodiments the anti-VEGF antibody comprises a CDRH1 comprising the following amino acid sequence: GYTFTNYGMN (SEQ ID NO: 1), a CDRH2 comprising the following amino acid sequence: WINTYTGEPTYADFKR (SEQ ID NO: 12), a CDRH3 comprising the following amino acid sequence: YPHYGS SHWYFDV (SEQ ID NO: 13), a CDRL1 comprising the following amino acid sequence: SASQDISNYLN (SEQ ID NO: 14), a CDRL2 comprising the following amino acid sequence: FTSSLHS (SEQ ID NO: 15) and a CDRL3 comprising the amino acid sequence: QQYSTVPWT (SEQ ID NO: 16).

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, I91, K101, 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 2μg/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 PBST, developed with 3,3', 5,5'-tetramethylbenzidine (TMB, Kirkegaard & Perry Labs, Gaithersburg, MD) substrate for approximately 5 min, quenched with 1.0 M H3PO4, and read spectrophotometrically at 450 nm. The ratio of IC50 values (IC50,ala/IC50,wt) represents the fold of reduction in binding affinity (the relative binding affinity).

(iii) **VEGF receptor molecules**

The two best characterized VEGF receptors are VEGFR1 (also known as Flt-1) and VEGFR2 (also known as KDR and FLK-1 for the murine homolog). The specificity of each receptor for each VEGF family member varies but VEGF-A binds to both Flt-1 and KDR. The full length Flt-1 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-1. A soluble form of the receptor exerts an inhibitory effect on the biological activity of the VEGF protein by binding to VEGF, thereby preventing it from binding to its natural receptors present on the surface of target cells. Also included are VEGF receptor fusion proteins, examples of which are described below.

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. W097/44453)

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.

IV. Therapeutic Uses

The present invention is based, in part, on the discovery that administration of an anti-NRP1 antibody to a patient in need thereof, e.g., a patient diagnosed with cancer, can result in adverse infusion reactions. This discovery was based on results
of Phase 1a and Phase 1b trials of anti-NRPl antibody MNRP1685A as described below in the Examples. Accordingly, the invention provides a method of treating a patient diagnosed with cancer, comprising administering to the patient premedication to prevent or ameliorate infusion reactions associated with administration of an anti-NRPl antibody, and then administering the anti-NRPl antibody. The method can further comprise administration of an anti-VEGF antibody, e.g., bevacizumab, and/or another anti-cancer agent such as a chemotherapeutic agent.

In some embodiments the premedication is administered to the patient the day before administration of the anti-NRPl antibody. For example, the patient is administered dexamethasone the night before administration of the anti-NRPl antibody. In some embodiments the premedication is administered to the patient the day before administration of the anti-NRPl antibody as well as the same day as administration of the anti-NRPl antibody. For example, the patient is administered dexamethasone the night before administration of the anti-NRPl antibody and the following day the patient is administered dexamethasone at least 60 minutes before administration of the anti-NRPl antibody. In another example, the patient is administered dexamethasone the night before administration of the anti-NRPl antibody and the following day the patient is administered one or more of the following compounds: dexamethasone, diphenhydramine, an H2 receptor antagonist, an H1 receptor antagonist, corticosteroids, non-steroidal anti-inflammatory drugs (NSAIDs) and acetaminophen, at least 60 minutes before administration of the anti-NRPl antibody. For example, the patient is administered 20 mg dexamethasone the day before administration of anti-NRPl antibody and is administered 10 mg dexamethasone, 50 mg of diphenhydramine, an H2 receptor antagonist and 650 mg of acetaminophen the same day as administration of the anti-NRPl antibody. In some

Each of the premedications administered to the patient may be dosed within the standard range for that premedication. Exemplary premedication used for preventing or ameliorating infusion reactions associated with administration of the anti-NRPl antibody are, for example, dexamethasone, diphenhydramine, an H2 receptor antagonist, an H1 receptor antagonist, acetaminophen, corticosteroids, and/or non-steroidal anti-inflammatory drugs (NSAIDs). Exemplary H2 receptor antagonists include cimetidine, ranitidine, famotidine and nizatidine.
The HI receptor antagonist may be selected from, but not limited to, anti-histamines. The corticosteroid may be selected from, but not limited to, glucocorticoids, mineralocorticoids, hydrocortisone, hydrocortisone acetate, cortisone acetate, tixocortol pivalate, prednisolone, methylprednisolone, prednisone, triamcinolone, acetonide, triamcinolone alcohol, mometasone, amcinonide, budesonide, desonide, fluocinonide, luocinolone acetonide, halcinonide, betamethasone, dexamethasone, dexamethasone sodium phosphate, fluocortolone, hydrocortisone-17-butyrate, hydrocortisone-17-valerate, aclometasone dipropionate, betamethasone valerate. Betamethasone dipropionate, clobetasone-17-butryate, clobetasol-17-propionate, fluocortolone caproate, fluocortolone pivalte and fluprednidene acetate. The NSAID maybe selected from, but not limited to, ibuprofen, naproxen, fenoprofen, ketoprofen, flurbiprofen, oxaprozin, indomethacin, sulindac, etodolac, diclofenac, piroxicam, meloxicam, droxicam, lornoxicam, isoxicam, mefenamic acid, meclofenamic acid, flufenamic acid and tolenamic acid.

Preferred dosages for the anti-NRPl antibody are in the range from about 1 lug/kg to about 50 mg/kg, preferably from about 5 mg/kg to about 40 mg/kg, preferably from about 24-33 mg/kg, including but not limited to 7.5 mg/kg, 10 mg/kg, 15 mg/kg, 20 mg/kg, 25 mg/kg, 30 mg/kg when given intravenously once every three weeks. The anti-NRPl antibody may also be given more frequently, such as every week or every two weeks.

Depending on the type and severity of the disease, preferred dosages for the anti-VEGF antibody are in the range from about 1 lug/kg to about 50 mg/kg, most preferably from about 5 mg/kg to about 15 mg/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.

The NRPl-specific antagonist may be administered as single agent. The invention also features the use of a combination of at least one NRPl-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 NRPI-specific antagonist is used in combination with one or more chemotherapeutic agents. 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 NRPI-specific antagonist with one or more chemotherapeutic agents (e.g., a cocktail). 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 NRPI-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 NRPI-specific antagonist. In this embodiment, the timing between at least one administration of the chemotherapeutic agent and at least one administration of the NRPI-specific antagonist is preferably approximately 1 month or less, and most preferably approximately 3 weeks, 2 weeks or less. Alternatively,
the chemotherapeutic agent and the NRP1 antagonist are administered concurrently to the patient, in a single formulation or separate formulations.

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. Variation in dosage will likely occur depending on the condition being treated. 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-NRP1 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-NRP1 antibody is co-administered with a growth inhibitory agent. For example, the growth inhibitory agent may be administered first, followed by the anti-NRP1 antibody. However, simultaneous administration or administration of the anti-NRP1 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-NRP1 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, 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). The anti-NRP1 antibody of the invention may be 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.

In addition to the above therapeutic regimes, the patient may be subjected to radiation therapy.

In certain embodiments, the administered anti-NRPl antibody is an intact, naked antibody. However, the anti-NRPl 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.

V. Dosages and Duration

The NRPl-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 NRPl-specific antagonist to be administered will be governed by such considerations, and is the minimum amount necessary to prevent, ameliorate, or treat, or stabilize the cancer. The NRPl-specific antagonist need not be, but is optionally, formulated with one or more agents currently used to prevent or treat cancer. The effective amount of such other agents depends on the amount of NRPl-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 NRPl-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, 5 mg/kg, 7.5 mg/kg, 10 mg/kg, 15 mg/kg, 20 mg/kg and 25 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 25 mg/kg, including but not limited to 5 mg/kg, 7.5 mg/kg, 10 mg/kg, 15 mg/kg, 20 mg/kg and 25 mg/kg. The progress of the therapy of the invention is easily monitored by conventional techniques and assays.

The NRPT-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, intracerebrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes. Local administration is particularly desired if extensive side effects or toxicity is associated with NRPT antagonism. An ex vivo strategy can also be used for therapeutic applications. Ex vivo strategies involve transfecting or transducing cells obtained from the subject with a polynucleotide encoding a NRPI 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 NRPI-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 NRP1-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 NRP1-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.

Alternatively, an inhibitory nucleic acid molecule or polynucleotide containing a nucleic acid sequence encoding a NRP1-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.

**VI. 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™, PLURONICS™ 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%.

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, 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-(methylenemacrylate) 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.

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 sulfo succinimid ester (conjugation through cysteine residues), N-hydroxy succinimide (through lysine residues), glutaraldehyde, succinic anhydride, SOCl₂, or R⁻N=C=NR, where R and R¹ 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 by Kohler et al, *Nature*, 256:495 (1975), or by recombinant DNA methods (U.S. Patent No. 4,816,567).

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-1 1 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.

In a further embodiment, antibodies or antibody fragments can be isolated from antibody phage libraries generated using the techniques described in McCafferty *et al.,* *Nature*, 348:552-554 (1990). Clackson *et al.,* *Nature*, 352:624-628 (1991) and Marks *et al,*

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

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

(iii) Humanized and human antibodies

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 (½) gene in chimeric and...

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

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

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

(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), Ile (I), Pro (P), Phe (F), Trp (W), Met (M)
2. uncharged polar: Gly (G), Ser (S), Thr (T), Cys (C), Tyr (Y), Asn (N), 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-acetylgalactosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxyllysine 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, W098/58964 (Raju, S.) and W099/22764 (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-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC). See Caron et al., J. Exp Med. 176:1 191-1 195 (1992) and Shopes, B. J. Immunol. 148:2918-2922 (1992).

Homodimeric antibodies with enhanced anti-tumor activity may also be prepared using heterobifunctional cross-linkers as described in Wolff et al. Cancer Research 53:2560-2565 (1993). Alternatively, an antibody can be engineered which has dual Fc regions and may thereby have enhanced complement lysis and ADCC capabilities. See Stevenson et al. Anti-Cancer Drug Design 3:219-230 (1989).

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 IgGl 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. 6,194,551B1, US Patent No. 6,242,195B1, US Patent No. 6,528,624B1 and US Patent No. 6,538,124 (Idusogie et al). The antibodies comprise an amino acid substitution at one or more of amino acid positions 270, 322, 326, 327, 329, 313, 333 and/or 334 of the Fc region thereof (Eu numbering of residues).

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., IgGl, 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 WOOO/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) *Immunconjugates*

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

Conjugates of the antibody and cytotoxic agent are made using a variety of bifunctional protein coupling agents such as N-succinimidy1-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaredehyde), 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-methyl diethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See W094/1 1026.

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

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-NRPI 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 premedication to prevent or ameliorate infusion reactions associated with administration of the anti-NRPI antibody prior to administering to a patient the anti-NRPI antibody. 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 NRPI-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 diagnosed with cancer, comprising a package, wherein the package comprises an anti-NRPI antibody
composition and instructions for using the anti-NRPI antibody composition in cancer therapy, wherein the instructions recite that the patient should be premedicated to ameliorate infusion reactions associated with administration of the anti-NRPI antibody. In some embodiments the premedication comprises dexamethasone. In some embodiments the premedication comprises one or more of the following compounds:
dexamethasone, diphenhydramine, an H2 receptor antagonist (e.g., cimetidine, ranitidine,
famotidine and nizatidine), an H1 receptor antagonist (e.g., anti-histamine),
corticosteroids, non-steroidal anti-inflammatory drugs (NSAIDs) and/or acetaminophen.

**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  Phase Ia trial of patients with locally advanced or metastatic solid
tumors treated with anti-NRPI antibody

This example concerns analysis of results obtained from patients with locally advanced or metastatic solid tumors treated with anti-NRPI antibody (MNRP1685A) in an open label, dose-escalation phase Ia trial. The primary objective of the study was to (1) evaluate the safety and tolerability of single and multiple dose of MNRP1685A administered by intravenous (IV) infusion every 3 weeks to patients with locally advanced or metastatic solid tumors and (2) estimate the maximum tolerated dose (MTD) and evaluate dose-limiting toxicities (DLTs) of MNRP1685A when administered by IV infusion every 3 weeks. The secondary goal of the study was to characterize the pharmacokinetics of MNRP1685A in patients with locally advanced or metastatic solid tumors.

Study Design

Figures 1 and 2 show summaries of the study design. The starting dose was 2 mg/kg MNRP1685A (Cohort 1) administered by IV infusion every 3 weeks. Dose levels of 5 mg/kg (Cohort 2), 10 mg/kg (Cohort 3), 15 mg/kg (Cohort 5), 20 mg/kg (Cohort 4), 30 mg/kg and 40 mg/kg MNRP1685A were also evaluated. The initial dose of MNRP1685A was given over 90 minutes followed by observation for 90 minutes. If the first infusion was well tolerated, subsequent dose could be given over ~30 minutes followed by a 30 minute observation period. No premedication was allowed for the first dose. Patients who experienced infusion-related symptoms could be premedicated with acetaminophen and/or diphenhydramine for subsequent infusions.

Eligible patients had an ECOG performance status of 0, 1 or 2, and histologic documentation of incurable, locally advanced or metastatic solid malignancy that failed to respond to at least one prior regimen or for which there is no standard therapy. Patients also had evaluable or measurable disease per RECIST or the following: prostate cancer patients with non-measurable disease were eligible if they had an increase in PSA level of >50% over baseline, the absolute increase was >5 ng/ml, and the increase was confirmed a second time; ovarian patients with non-measurable disease were eligible if they had an increase in CA-125
of >2x baseline (or >2x ULN in case of prior normal CA-125 level) and the increase was confirmed a second time.

Patients were excluded if they had inadequate hematologic and organ function, defined by the following (hematological parameters were assessed ≥14 days after a prior transfusion, if any): granulocyte count <1000/µL, hemoglobin <9 g/dL, platelet count <100,000/µL, total bilirubin >1.5 x ULN (with the following exception: patients with known Gilbert’s disease who have serum bilirubin <3 x ULN could be enrolled), AST and ALT >2.5 x ULN (with the following exception: patients with documented liver metastases may have AST and/or ALT ≤5 x ULN), alkaline phosphatase >2.5 x ULN (with the following exception: patients with documented liver or bone metastases may have alkaline phosphatase ≤5 x ULN), serum creatine >1.5 x ULN (with the following exception: creatine clearance of >50 mL/min based on a 24 hour urine collection was documented), international normalized ration (INR) >1.5 x ULN or activated partial thromboplastin time (aPTT) >1.5 x ULN.

Patients were also excluded if they had any of the following: anti-cancer therapy within 4 weeks prior to initiation of the current study treatment with the following exceptions: hormonal therapy with gonadotropin-releasing hormone agonists or antagonists for prostate cancer, hormone replacement therapy or oral contraceptives, or palliative radiation to bone metastases >2 weeks prior to Day 1 of study treatment.

Patients were also excluded if they had adverse events from prior anti-cancer therapy that had not resolved to Grade <1, except for alopecia; leptomeningeal disease as the only manifestation of the current malignancy; active infection requiring IV antibiotics; active autoimmune disease that was not controlled by non-steroidal anti-inflammatory drugs, inhaled steroids, od the equivalent of prednisone 5 mg/day or less; bisphosphonate therapy for symptomatic hypercalcemia; known clinically significant history of liver disease, including viral or other hepatits, current alcohol abuse, or cirrhosis; known HIV infection; known primary CNS malignancy or untreated/active CNS metastases; pregnancy or lactation; inadequately controlled hypertension; prior history of hypertensive crisis or hypertensive encephalopathy; NYHA Class II or greater congestive heart failure; history of myocardial infarction or unstable angina within 6 months prior to Day 1 of current study treatment; history of stroke or transient ischemic attack within 6 months prior to Day 1 of current study treatment; significant vascular disease within 6 months prior to Day 1 of current study treatment;
treatment; history of hemoptysis within 1 month prior to Day 1 of current study treatment; evidence of bleeding diathesis or significant coagulopathy (in the absence of therapeutic anticoagulation); major surgical procedure, open biopsy or significant traumatic injury within 28 days prior to Day 1 of current study treatment; core biopsy or other minor surgical procedure, excluding placement of vascular access device, within 7 days prior to Day 1 of current study treatment; incisions from any procedure that are not fully healed on Day 1 of current study treatment; serious, non-healing wound, active ulcer, or untreated bone fracture; protenuria at screening as demonstrated by a urine protein creatinine (UPC) ratio of ≥1.0; or intrathoracic lung carcinoma of squamous cell histology.

All patients were carefully followed for adverse events during the study and for 30 days after the last dose of MNRP1685A. Adverse events were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE). The dose-limiting toxicity (DLT) Assessment Window was 21 days following the first dose of MNRP1685A (Days 1-21 of Cycle 1). DLTs were defined as follows: grade ≥ 3 non-hematologic or non-hepatic toxicity (exceptions: hypertension, proteinuria, acute infusion reaction), grade ≥ 3 hepatic transaminase or alkaline phosphatase, and select bevacizumab-related grade ≥ 1 or 2 adverse events (e.g., pulmonary or CNS hemorrhage, ATE, gastrointestinal perforation, RPLS). Continued dosing with MNRP1685A every three weeks for a maximum of 16 cycles or 1 year (whichever occurred first) was offered to patients who derived ongoing benefit and who did not experience significant toxicity.

Results

Figure 3 summarizes patient characteristics. Figure 9 shows how long the patients were on study. Figures 4 and 5 show pharmacokinetics of the anti-NRPI antibody. Figure 8 shows that Placental Growth Factor (P1GF) is a pharmacodynamic marker of systemic NRPI pathway inhibition.

Dose levels 2, 5 and 10 mg/kg were cleared without dose-limiting toxicities (DLTs). In Cohort 4 (20 mg/kg MNRP1685A), one patient experienced a Grade 2 acute infusion reaction (fatigue, puritus, hypotension, rigors and dizziness) with the first MNRP1685A infusion and a DLT of Grade 3 upper gastrointestinal hemorrhage on Day 5 of Cycle 1.

Another patient of Cohort 4 had a Grade 2 acute infusion reaction (fatigue, pyrexia, dizziness, unsteadiness on feet, and somnolence). Acute infusion reactions with less significant
symptoms were also observed at lower dose levels. Four of 10 patients in Cohorts 1 to 3 experienced a Grade 1 acute infusion reaction with the first MNRP1685A infusion, and Grade 2 acute infusion reactions were seen in 2 of 10 patients. One patient in Cohort 3 had to be replaced because the patient did not receive the full assigned dose of MNRP1685A as a result of a Grade 2 acute infusion reaction (hypertension, pyrexia, rigors, and hypoxia). A second patient in Cohort 3 experienced a Grade 2 acute infusion reaction (diaphoresis, rigors, hypertension, nausea, and hypotension) despite institution of mandatory premedication with diphenhydramine and acetaminophen. Treatment-related adverse events are also shown in Figure 6.

Because of the significant incidence of acute infusion reactions seen in patients, management of acute infusion reactions at an intermediate dose level (15 mg/kg) was investigated. The first patient at 15 mg/kg was premedicated with diphenhydramine, acetaminophen, ibuprofen, and cimetidine, and the MNRP1685A infusion was given over 120, instead of 90, minutes. This patient experienced a Grade 2 acute infusion reaction with headache, fatigue, and hypotension. All subsequent patients were premedicated with 10 mg of dexamethasone IV. The first patient with dexamethasone premedication had a Grade 2 acute infusion reaction, but with mild and short-lived symptoms (vasovagal episode, urticaria, pruritus, and dizziness). The second patient with dexamethasone premedication had a Grade 1 acute infusion reaction with a brief episode of bilateral pruritic rash below the knees. Figure 7 summarizes data of infusion-related symptoms with and without dexamethasone premedication for Cycle 1.

Example 2 Phase 1b trial of patients with locally advanced or metastatic solid tumors treated with anti-NRPI antibody

This example concerns analysis of results obtained from patients with locally advanced or metastatic solid tumors treated with anti-NRPI antibody (MNRP1685A) in combination with bevacizumab (Arm A) or in combination with bevacizumab and paclitaxel (Arm B) in an open label, dose-escalation phase 1b trial. The primary objectives of the study were to evaluate safety and tolerability of MNRP1685A (e.g., identify incidence and nature of DLTs) administered in combination with bevacizumab with or without paclitaxel, estimate the maximum tolerated dose and identify a recommended Phase II dose of anti-NRPI when combined with bevacizumab with or without paclitaxel. The secondary objective was to
characterize pharmacokinetics of MNRP1685A and bevacizumab when administered in combination. Plasma samples were used to measure placental growth factor (PIGF), basis fibroblast growth factor (bFGF), and other circulating factors for exploratory analyses and for comparison to results of the phase 1a study described in Example 1 above.

5 Study Design

Arm A: The starting dose was 7.5 mg/kg MNRP1685A administered in combination with 15 mg/kg of bevacizumab every three weeks. Doses of 7.5, 15, 24 and 36 mg/kg MNRP1685A in combination with 15 mg/kg of bevacizumab every three weeks were also evaluated.

Arm B: MNRP1685A and bevacizumab (10 mg/kg) were administered on Days 1 and 15 and paclitaxel (90 mg/m²) intravenously on Days 1, 8 and 15 of 4-week cycles. Three dose escalation cohorts (12, 16 and 24 mg/kg) of MNRP1685A were evaluated.

Exclusion and Inclusion Criteria for patients enrolled in this study:

Inclusion Criteria:

• Adequate hematologic and end organ function
• Evaluable disease or measurable disease per Response Evaluation Criteria In Solid Tumors (RECIST)
• Agreement to use an effective form of contraception for the duration of the study

Inclusion Criteria Unique to Arm A:

• Histologically or cytologically documented, incurable, locally advanced, or metastatic solid malignancy that has progressed on, or failed to respond to, at least one prior regimen

Inclusion Criteria Unique to Arm B:

• Histologically or cytologically documented, incurable, locally advanced, or metastatic solid malignancy; a maximum of two prior chemotherapy regimens is allowed

Exclusion Criteria:

• Any anti-cancer therapy, including chemotherapy, hormonal therapy, or radiotherapy, within 3 weeks prior to initiation of study treatment with the following exceptions: hormonal therapy with gonadotropin-releasing hormone (GnRH) agonists or antagonists for prostate cancer; herbal therapy > 1 week prior to Day 1; hormone-replacement therapy or oral...
contraceptives; palliative radiotherapy for bone metastases > 2 weeks prior to Day 1

- Leptomeningeal disease as a manifestation of the current malignancy
- Active infection requiring IV antibiotics

5

- Active autoimmune disease that is not controlled by nonsteroidal anti-inflammatory drugs, inhaled corticosteroids, or the equivalent of ≤ 10 mg/day prednisone
- Bisphosphonate therapy for symptomatic hypercalcemia
- Known clinically significant liver disease, including active viral, alcoholic, or other hepatitis, or cirrhosis

10

- Known primary CNS malignancy, or untreated or active CNS metastases
- Pregnancy, lactation or breast feeding
- Inadequately controlled hypertension

15

- History of hypertensive crisis or hypertensive encephalopathy
- History of myocardial infarction or unstable angina within 6 months prior to Day 1
- New York Heart Association (NYHA) Class II or greater CHF
- History of stroke or transient ischemic attack (TIA) within 6 months prior to Day 1

20

- Significant vascular disease (e.g., aortic aneurysm requiring surgical repair or recent peripheral arterial thrombosis) within 6 months prior to Day 1
- History of hemoptysis within 1 month prior to Day 1
- Evidence of bleeding diathesis or significant coagulopathy in the absence of stable therapeutic anticoagulation

25

- Major surgical procedure, open biopsy, or significant traumatic injury within 28 days prior to Day 1 or anticipation of need for major surgical procedure during the course of the study
- Core biopsy or other minor surgical procedure, excluding placement of a vascular access device, within 7 days prior to Day 1
- History of abdominal fistula, gastrointestinal perforation or intra-abdominal abscess within 6 months prior to Day 1
• Clinical signs or symptoms of gastrointestinal obstruction or requirement for parenteral hydration, parenteral nutrition, or tube feeding
• Evidence of abdominal free air not explained by paracentesis or recent surgical procedure
• Serious, non-healing wound, active gastrointestinal ulcer, or untreated bone fracture
• Intrathoracic lung carcinoma of squamous cell histology
• Grade ≥ 2 sensory neuropathy
• Any other disease, metabolic dysfunction, physical examination finding, or clinical laboratory finding that, in the investigator's opinion, gives reasonable suspicion of a disease or condition that contraindicates the use of an investigational drug or that may affect the interpretation of the results or render the patient at high risk from treatment complications
• Known hypersensitivity to recombinant human antibodies

Exclusion Criterion Unique to Arm B:
• Known significant hypersensitivity to paclitaxel or other drugs using the vehicle cremophor

All patients in Arms A and B received premedication, which included dexamethasone, for the first MNRP1685A infusion. Specifically, at bedtime the night before the first infusion of MNRP1685A the patient was administered 20 mg dexamethasone orally (PO). On Day 1, at least 60 minutes before MNRP1685A infusion, the patient was administered 10 mg dexamethasone IV (20 mg dexamethasone IV could be given for MNRP1685A dose levels >15 mg/kg), 50 mg of diphenhydramine IV (diphenhydramine could also be given PO and/or at 25 mg), H2 receptor antagonist (e.g., cimetidine, ranitidine, famotidine and nizatidine) and 650 mg of acetaminophen PO. The need for premedication was assessed before each subsequent MNRP1685A infusion and premedication was adjusted accordingly. Discontinuation of premedication was considered if the prior MNRP1685A infusion resulted in Grades ≤ 1 acute infusion reaction. If the patient experienced an infusion-associated adverse event, premedication for MNRP1685A could be modified or introduced for the next MNRP1685A infusion, and/or the infusion time may be extended. In Arm B, all patients received standard premedication for the paclitaxel infusion (10 mg dexamethasone IV, 50 mg diphenhydramine IV, and 300 mg cimetidine IV or another H2 receptor antagonist 30-60 minutes prior to paclitaxel administration). Premedication for paclitaxel and the paclitaxel
infusion occurred prior to the MNRP1685A infusion. Thus, paclitaxel premedication also served as premedication for the MNRP1685A infusion. In some cases, after receiving the paclitaxel premedication patients received additional premedication specifically for MNRP1685A with acetaminophen, ibuprofen, diphenhydramine and/or cimetidine or another H2 receptor antagonist.

All patients were carefully followed for adverse events during the study and for 30 days after the last dose of study treatment or until initiation of another anti-cancer therapy, whichever occurred first. Adverse events were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE). The dose-limiting toxicity (DLT) Assessment Window was 21 days (Days 1 to 21 of Cycle 1) for Arm A and 28 days (Days 1 to D28 of Cycle 1) for Arm B following the first dose of MNRP1685A. Continued treatment with MNRP1685A with bevacizumab and paclitaxel (if applicable) for up to 1 year was offered to patients who derived ongoing benefit and who did not experience significant toxicity.

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 treating a patient diagnosed with cancer, comprising administering to the patient premedication to ameliorate infusion reactions associated with administration of an anti-NRPI antibody, and administering an effective amount of an anti-NRPI antibody.

2. The method of claim 1, wherein the method further comprises increasing infusion time of administering the anti-NRPI antibody to the patient.

3. The method of claim 1, wherein the method further comprises administering an effective amount of anti-VEGF antibody to the patient.

4. The method of claim 1, wherein the method further comprises administering a chemotherapeutic agent to the patient.

5. The method of claim 1, wherein the cancer is colorectal cancer, breast cancer, lung cancer, renal cancer, ovarian cancer or glioblastoma.

6. The method of claim 1, wherein the premedication comprises administration of dexamethasone.

7. The method of claim 6, wherein the premedication comprises administration of one or more of the following compounds: dexamethasone, diphenhydramine, H1 receptor antagonist, H2 receptor antagonist and/or acetaminophen.

8. The method of claim 7, wherein the H2 receptor antagonist is selected from the group consisting of cimetidine, ranitidine, famotidine and nizatidine.

9. The method of claim 6, wherein the dexamethasone is administered to the patient the day before administration of the anti-NRPI antibody.

10. The method of claim 9, further comprising administering dexamethasone to the patient the same day as administration of the anti-NRPI antibody.

11. The method of claim 10, wherein the patient is administered 20 mg dexamethasone the day before administration of anti-NRPI antibody and wherein the patient is administered 10 mg dexamethasone, 50 mg of diphenhydramine, an H2 receptor antagonist and 650 mg of acetaminophen the same day as administration of the anti-NRPI antibody.

12. The method of any one of claims 1-11, wherein the anti-NRPI antibody is MNRP1685A.
13. The method of any one of claims 1-11, wherein the anti-NRPl antibody competes with MNRP 1685A for binding to NRP 1.

14. The method of any one of claims 1-11, wherein the anti-NRPl antibody binds to the same epitope as MNRP 1685A.

15. The method of any one of claims 1-11, wherein the anti-NRPl antibody comprises a CDRH1 comprising the amino acid sequence of SEQ ID NO:3, a CDRH2 comprising the amino acid sequence of SEQ ID NO:4, a CDRH3 comprising the amino acid sequence of SEQ ID NO:5, a CDRL1 comprising the amino acid sequence of SEQ ID NO: 6, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 7, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 8.

16. The method of any one of claims 1-11, wherein the anti-NRPl antibody has a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 1, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 2.

17. A kit for treating a patient diagnosed with cancer, comprising a package, wherein the package comprises an anti-NRPl antibody composition and instructions for using the anti-NRPl antibody composition in cancer therapy, wherein the instructions recite that the patient should be premedicated to ameliorate infusion reactions associated with administration of the anti-NRPl antibody.
Study Design

Eligibility Criteria

- ECOG 0-2
- Advanced solid tumors
- Adequate hematologic and organ function
- No active CNS metastases
- No condition causing bevacizumab ineligibility

Dose Limiting Toxicities

- Grade ≥ 3 non-hematologic or non-hepatic toxicity
  - Key exceptions: HTN, proteinuria, acute infusion reaction
- Grade ≥ 3 hepatic transaminases or alkaline phosphatase
- Select bevacizumab-related Grade ≥ 1 or 2 AEs
  - (e.g. pulmonary or CNS hemorrhage, ATE, GI perforation, RPLS)

FIG. 1

Study Design

- Multicenter, Phase 1 study
- Anti-NRP1 administered IV over 90 minutes every 3 weeks
- Increasing dose cohorts of 3 - 6 patients
- Dose escalation based on observed toxicity

FIG. 2
### Patient Characteristics

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<th>Category</th>
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</tr>
<tr>
<td>Pancreas</td>
<td>2</td>
</tr>
<tr>
<td>Ovarian</td>
<td>2</td>
</tr>
<tr>
<td>Cervical</td>
<td>2</td>
</tr>
<tr>
<td>Neuroendocrine</td>
<td>2</td>
</tr>
<tr>
<td>Others</td>
<td>13</td>
</tr>
</tbody>
</table>

ECOG, Eastern Cooperative Oncology Group, n = number of patients
Data cut-off 16 Feb 2010

**FIG. 3**
Anti-NRP1 Pharmacokinetics

- Non-linear clearance (CL) observed over the entire dose range
  - Significant target-mediated CL consistent with broad NRP1 tissue expression

**FIG. 4**
### Anti-NRP1 Pharmacokinetics

<table>
<thead>
<tr>
<th>Dose mg/kg</th>
<th>n</th>
<th>$C_{\text{max}}$ (μg/mL) (mean±SD)</th>
<th>$AUC_{\text{inf}}$ (day•μg/mL) (mean±SD)</th>
<th>CL (mL/day/kg) (mean±SD)</th>
<th>$V_{\text{ss}}$ (mL/kg) (mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>3</td>
<td>34.5 ± 13.5</td>
<td>40.8 ± 17.2</td>
<td>54.5 ± 19.4</td>
<td>42.3 ± 13.9</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>94.2 ± 18.6</td>
<td>176 ± 70.5</td>
<td>51.3 ± 25.1</td>
<td>77.7 ± 28.2</td>
</tr>
<tr>
<td>10</td>
<td>3</td>
<td>199 ± 60.7</td>
<td>447 ± 192</td>
<td>26.2 ± 14.6</td>
<td>64.8 ± 19.4</td>
</tr>
<tr>
<td>15</td>
<td>6</td>
<td>366 ± 74.6</td>
<td>1304 ± 316</td>
<td>12.1 ± 3.1</td>
<td>42.3 ± 19.2</td>
</tr>
<tr>
<td>20</td>
<td>6</td>
<td>468 ± 107</td>
<td>1627 ± 782</td>
<td>14.2 ± 5.4</td>
<td>63.6 ± 30.1</td>
</tr>
<tr>
<td>30</td>
<td>3</td>
<td>532 ± 154</td>
<td>2143 ± 871</td>
<td>16.4 ± 8.2</td>
<td>89.1 ± 29.3</td>
</tr>
<tr>
<td>40</td>
<td>3</td>
<td>1007 ± 523</td>
<td>4660 ± 1280</td>
<td>9.2 ± 2.6</td>
<td>73.3 ± 34.3</td>
</tr>
</tbody>
</table>

### FIG. 5

#### Treatment-related Adverse Events

Grade ≥3 AEs

<table>
<thead>
<tr>
<th>Event Term</th>
<th>Cohort</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 3 Hemorrhage, upper gastrointestinal</td>
<td>20 mg/kg</td>
<td>Dose-limiting toxicity, resolved</td>
</tr>
<tr>
<td>Grade 3 Thrombocytopenia</td>
<td>30 mg/kg</td>
<td>Coincided with Grade 3 fungemia, resolved</td>
</tr>
</tbody>
</table>

Grade 1 and 2 AEs occurring in >1 patient (≥5%)

<table>
<thead>
<tr>
<th>Event Term</th>
<th>Total</th>
<th>Grade 1</th>
<th>Grade 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute Infusion Reaction</td>
<td>17 (61%)</td>
<td>1</td>
<td>16</td>
</tr>
<tr>
<td>Fatigue</td>
<td>4 (14%)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Myalgia</td>
<td>2 (7%)</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Common Toxicity Criteria: CTCAE 3.0

Data cut-off 16 Feb 2010

### FIG. 6
Infusion-related Symptoms with and without Dexamethasone Premedication
Analysis for Cycle 1 Only

% of Patients

- Pyrexia
- Chills
- Headache
- Hypotension
- Hypertension
- Nausea
- Fatigue
- Dizziness
- Balance Disorder
- Somnolence
- Myalgia
- Malaise
- Vomiting
- Hyperhidrosis
- Hypoxia
- Presyncope
- Diarrhea
- Chest Discomfort
- Blurred Vision
- Urticaria
- Tachycardia
- Rash
- Pruritus

No dexamethasone premedication (n=13)
Dose cohorts 2, 5 and 10 mg/kg,
1 patient at 15 mg/kg, 2 patients at 20 mg/kg

Dexamethasone premedication (n=15)
Dose cohorts 15 mg/kg (except for 1),
20 mg/kg (except for 2), 30 mg/kg and 40 mg/kg

Premedication in both groups (except for dose cohorts 2 and 5 mg/kg and 1 patient at 10 mg/kg): Diphenhydramine, H2 receptor antagonist of choice, acetaminophen

FIG. 7
Data cut-off 16 Feb 2010; patients off study for progressive disease unless noted otherwise
AIR; Acute Infusion Reaction; UGI, Upper Gastrointestinal

FIG. 9
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C07K 16/00 A61K 39/00 (201 1.01)
USPC - 514/389.7: 424/142.1

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)

USPC - C07K 1600; A61K 3900 (201 1.01)
USPC - 514/389.7: 424/142.1

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

USPC - 514/388.15; 530/388.15 (Text Search)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PubWEST (PGPB, USPT, USOC, EPAB, JPAB); Google Scholar; Google: antibody, mAb, cancer, tumor, neoplastS, neuropilin-1, NRPI, NRP-1, dexamethasone, infus$, reaction, side effect, infusion, time, rate, slow, slowly, infus$, MNRP1685A.

GenCore 6.3: SEQ ID NO:1-8

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>US 2008/0213268 A1 (WATTS et al.) 04 September 2008 (04.09.2008) SEQ ID NOs: 5-6, 129, 131-134; para [0028], [0067], [0090], [0161]-[0165], [0167], [0170]-[0171], [0190], [0191]</td>
<td>1-14 and 17</td>
</tr>
<tr>
<td>Y</td>
<td>A Study of MNRP1685A in Combination With Bevacizumab With or Without Paclitaxel in Patients With Locally Advanced or Metastatic Solid Tumors. 06 August 2009 [Retrieved from the Internet 19 July 2011 &lt;URL: <a href="http://clinicaltrials.gov/archive/NCT00954642/2009_08_06%3E">http://clinicaltrials.gov/archive/NCT00954642/2009_08_06&gt;</a>]; pg 1</td>
<td>12-14</td>
</tr>
</tbody>
</table>

Further documents are listed in the continuation of Box C. |

* "A" document defining the general state of the art which is not considered to be of particular relevance

** "E" earlier application or patent but published on or after the international filing date

*** "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

Date of the actual completion of the international search 19 July 2011 (19.07.2011)

Date of mailing of the international search report 08 AUG 2011

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