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(54) Title: TREATING ANEMIA BY INHIBITION OF VEGF

(57) Abstract: Methods of treating anemic disorders in human subjects comprising administering a VEGF antagonist comprising a fusion polypeptide having an immunoglobulin-like (Ig) domain 2 of the VEGF receptor FItI and Ig domain 3 of the VEGF receptor FIkI or Flt4, and a multimerizing component. Further included are methods of preventing anemia, increasing hematocrit levels, and increasing or stimulating erythropoietin, particularly in subjects being treated for cancer with chemotherapeutic agents and/or radiation.

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TREATING ANEMIA BY INHIBITION OF VEGF

BACKGROUND

Field of the Invention

[0001] The field of the invention is generally related to methods of treating anemia with an agent capable of blocking, inhibiting, or ameliorating vascular endothelial growth factor (VEGF).

Description of Related Art

[0002] The production of red blood cells in mammals, erythropoiesis, is under the control of the hormone erythropoietin (EPO). EPO is normally present in low concentrations in plasma, where it is sufficient to maintain equilibrium between normal blood cell loss (i.e., through aging) and red blood cell production.

[0003] Anemia is a decrease in red blood cell mass caused by decreased production or increased destruction of red blood cells. EPO is currently used for treatment of the anemias associated with end-stage renal failure, acquired immunodeficiency syndrome (AIDS), and anemia associated with cancer chemotherapy. See for example, US 5,846,528. Other groups of anemic disorders, resulting from an inherited abnormality in globin production, are hemoglobinopathies, including thalassemias, results from inherited defects in the rate of synthesis of one or more of the globin chains.

[0004] US 2004/0115166 describes the treatment of autoimmune hemolytic anemia with a inhibitor of VEGF-B. US 2003/0175276 describes a method of inhibiting angiogenesis with an anti-VEGF antibody for the treatment of sickle cell anemia.

BRIEF SUMMARY OF THE INVENTION

[0005] In a first aspect, the invention features a method of treating an anemic disorder comprising administering to a human subject a vascular endothelial growth factor (VEGF) antagonist capable of blocking, inhibiting, or ameliorating VEGF-mediated activity. More specifically, the VEGF antagonist a high affinity fusion protein dimer (or "trap") comprising a fusion polypeptide having an immunoglobulin-like (Ig) domain 2 of the VEGF receptor Flt1 and Ig domain 3 of the VEGF receptor Flk1 or Flt4, and a multimerizing component. Even more specifically, the VEGF antagonist comprises a fusion polypeptide selected from the group consisting of acetylated Flt-1(1-3)-Fc, Flt-1(1-$3_{R->N}$)-Fc, Flt-1(1-$3_{\Delta B}$)-Fc, Flt-1(2-$3_{\Delta B}$)-Fc, Flt-1(2-3)-Fc, Flt-1D2-VEGFR3D3-FcΔC1(a), Flt-1D2-Flk-1D3-FcΔC1(a), and VEGFR1R2-FcΔC1(a). In a preferred embodiment, the VEGF antagonist is VEGFR1R2-FcΔC1(a) (SEQ ID NO:4).

[0006] The anemic disorder treated by the method of the invention is selected from the group consisting of anemia associated with chronic renal failure, cancer, cancer chemotherapy and antiviral therapy. The anemic disorder may also be nutritional iron-deficiency (hypoferric) anemia,

blood-loss (hemorrhagic) anemia, or hemolytic anemia. Preferably, the subject treated by the methods of the invention has been or will be treated with chemotherapy or radiation.

[0007] Administration of the agent may be by any method known in the art, including subcutaneous, intramuscular, intradermal, intraperitoneal, intravenous, intranasal, or oral routes of administration. Administration may be by single or multiple doses.

[0008] In a second aspect, the invention features a method of preventing anemia resulting from or expected to result from cancer chemotherapy in a subject at risk, comprising administering to a human subject a vascular endothelial growth factor (VEGF) antagonist capable of blocking, inhibiting, or ameliorating VEGF-mediated activity, wherein the VEGF antagonist is a fusion polypeptide consisting of an immunoglobulin-like (Ig) domain 2 of the VEGF receptor Flt1 and Ig domain 3 of the VEGF receptor Flk1 or Flt4, and a multimerizing component. In a preferred embodiment, the VEGF antagonist is VEGFR1R2-FcΔC1(a) (SEQ ID NO:4). In a preferred embodiment, the subject treated is a subject undergoing or who will undergo treatment with radiation and/or chemotherapy.

[0009] In a third aspect, the invention features a method of increasing hematocrit level in a subject in need thereof, comprising administering to a human subject a vascular endothelial growth factor (VEGF) antagonist comprising a fusion polypeptide having an immunoglobulin-like (Ig) domain 2 of the VEGF receptor Flt1 and Ig domain 3 of the VEGF receptor Flk1 or Flt4, and a multimerizing component. In a preferred embodiment, the VEGF antagonist is VEGFR1R2-FcΔC1(a) (SEQ ID NO:4). In one embodiment, the subject treated has been determined to have a hematocrit level of less than abou t 33% of total blood volume, e.g., is diagnosed as anemic. The VEGF antagonist of the invention is administered in a therapeutically effective amount sufficient to produce an increase in hematocrit above 33% of total blood volume.

[0010] In a fourth aspect, the invention features a method of increasing erythropoietin levels in a subject in need thereof, comprising administering to a human subject a vascular endothelial growth factor (VEGF) antagonist comprising a fusion polypeptide having an immunoglobulin-like (Ig) domain 2 of the VEGF receptor Flt1 and Ig domain 3 of the VEGF receptor Flk1 or Flt4, and a multimerizing component.

[0011] In a fourth aspect, the invention features a method of stimulating erythropoiesis in a subject in need thereof, comprising administering to a human subject a vascular endothelial growth factor (VEGF) antagonist comprising a fusion polypeptide having an immunoglobulin-like (Ig) domain 2 of the VEGF receptor Flt1 and Ig domain 3 of the VEGF receptor Flk1 or Flt4, and a multimerizing component.

[0012] Other objects and advantages will become apparent from a review of the ensuing detailed description.

[0013] In a fifth aspect, the invention encompasses the use of an agent capable of blocking,

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inhibiting, or ameliorating vascular endothelial growth factor (VEGF)-mediated activity in the preparation of a medicament for: (a) the treatment of an anemic disorder in a mammal; (b) preventing anemia expected to result from cancer treatment in a subject at risk thereof; (c) increasing hematocrit level in a subject in need thereof; or (d) increasing erythropoietin levels or stimulating erythropoiesis in a subject in need thereof. In specific embodiments, the anemic disorder is characterized by a reduced hematocrit level and/or associated with chronic renal failure, cancer, cancer chemotherapy, radiation treatment, antiviral therapy, nutritional iron-deficiency, blood-loss, or hemolysis. The anemic disorder may result from treatment with chemotherapy or radiation. In one embodiment for increasing hematocrit level, the hematocrit level in a subject in need thereof is less than about 33% of total blood volume and administration of the fusion polypeptide is in an amount sufficient to increase the hematocrit level above 33% of total blood volume. In all embodiments, the preferred agent capable of blocking, inhibiting, or ameliorating VEGF activity is a VEGF antagonist VEGFR1R2-FcΔC1(a) (SEQ ID NO:4). In any of the uses of the invention, administration may be subcutaneous or intravenous, and may be provided in a single dose or multiple doses.

BRIEF DESCRIPTION OF THE FIGURES

[0014] Fig. 1 is a bar graph of percent hematocrit increase in SCID mice (n=3-4 per group) treated with cumulative weekly doses of 5, 50, or 75 mg/kg VEGF trap protein (SEQ ID NO:4) delivered by subcutaneous injection. Hematocrit was measured at 8 and 9.5 weeks,. Control hematocrit data (0 ug/kg) is based on data obtained from mice sacrificed at 8 weeks.

[0015] Fig. 2 is a graph showing the effect of varying amounts of VEGF trap administered over an 8 week period by subcutaneous twice weekly injection to mice with the indicated doses being the cumulative weekly dose (◆= 0 mg/kg; ■= 5 mg/kg; ▲=50 mg/kg). Hematocrit at day 0 is based on historical hematocrit data from mice of the same strain and approximate age.

[0016] Fig. 3 is a bar graph of changes in hematocrit observed at 13 weeks in monkeys (n=6) treated biweekly with 0 (control), 15 or 30 mg/kg of VEGF trap protein.

DETAILED DESCRIPTION

[0017] Before the present methods are described, it is to be understood that this invention is not limited to particular methods, and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only the appended claims.

[0018] As used in this specification and the appended claims, the singular forms "a", "an", and "the" include plural references unless the context clearly dictates otherwise. Thus for example, a

reference to "a method" includes one or more methods, and/or steps of the type described herein and/or which will become apparent to those persons skilled in the art upon reading this disclosure and so forth.

[0019] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described

Definitions

[0020] By the term "therapeutically effective dose" is meant a dose that produces the desired effect for which it is administered. The exact dose will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques (see, for example, Lloyd (1999) The Art, Science and Technology of Pharmaceutical Compounding).

[0021] By the term "blocker", "inhibitor", or "antagonist" is meant a substance that retards or prevents a chemical or physiological reaction or response. Common blockers or inhibitors include, but are not limited to antisense molecules, antibodies, antagonists and their derivatives. More specifically, an example of a VEGF antagonist is, for example, an anti-VEGF antibody, or a VEGF-binding receptor-based fusion protein ("trap") such as VEGFR1R2-FcΔC1(a) (SEQ ID NOs:1-2). For a complete description of VEGF-receptor based antagonists including VEGFR1R2-FcΔC1(a), see PCT publication WO/00/75319.

[0022] The term "erythropoiesis" denotes the proliferation and/or differentiation of erythroid precursor cells. Standard measures of erythroid cell proliferation and differentiation include hematocrit and reticulocyte counts. Hematocrit is a measurement of red blood cells, and is commonly expressed as the percentage of total blood volume which consists of erythrocytes. Reticulocyte counts measure 1-2 day-old cells that contain mRNA (absent in mature erythrocytes) and aggregates of ribosomes as demonstrated by staining (Erslev, A., "Reticulocyte Enumeration", in Hematology, McGraw-Hill, NY, 1990). A reticulocyte count is the percentage of such cells per 500 or 1000 cells counted. An average range for reticulocyte counts is 0.8% to 1.2%. EPO is commercially available (R & D Systems, Minneapolis, Minn. and Amgen, Thousand Oaks, Calif.) and activity is measured by calibration against the second international reference preparation of erythropoietin (Annable et al. (1972) Bull. Wld. Hlth. Org. 47:99) using an *in vivo* assay which measures the incorporation of ^{56}Fe into red blood cells of exhypoxic polycythemic mice (Cotes et al. (1961) Nature 191:1065) or by *in vitro* cell proliferation assay that uses a factor-dependent human erythroleukemic cell line, TF-1 (Kitamura et al. (1989) J. Cell. Physiol. 140:323). See also, US 6,099,830.

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General Description

[0023] Anemia is a deficiency in the production of red blood cells (erythrocytes) resulting in a reduction in the level of oxygen transported by blood to the tissues of the body. Hypoxia may be caused by loss of large amounts of blood through hemorrhage, destruction of red blood cells from exposure to autoantibodies, radiation or chemicals, or reduction in oxygen intake due to high altitudes or prolonged unconsciousness. When hypoxia is present in tissue, EPO production is stimulated and increases red blood cell production. EPO promotes the conversion of primitive precursor cells in the bone marrow into pro-erythrocytes which subsequently mature, synthesize hemoglobin and are released into the circulation as red blood cells. When the number of red blood cells in circulation is greater than needed for normal tissue oxygen requirements, the level of EPO in circulation is decreased.

[0024] Severe reductions in both megakaryocyte and erythrocyte levels can be associated with the treatment of various cancers with chemotherapy and radiation and diseases such as AIDS, aplastic anemia and myelodysplasias. Levels of megakaryocytes and/or erythrocytes that become too low, for example, platelet counts below 25,000 to 50,000 and hematocrits of less than 25%, are likely to produce considerable morbidity and in certain circumstances these levels are life-threatening. In addition to treating the underlying disease, specific treatments include platelet transfusions for thrombocytopenia (low platelet counts) and stimulation of erythropoiesis using EPO or transfusion of red blood cells for anemia.

[0025] As shown in the experimental section below, it is now shown that administration of a VEGF antagonist results in an increased hematocrit. Previous studies (Gerber et al. (1999) Development 126:1149-1159) reported that VEGF blockade in developing mice caused an increase in hematocrit as well as in levels of erythropoietin message in kidney; however, it was suggested that these changes were side effects of cardiac and/or pulmonary hypoplasia. A small molecule inhibitor of the VEGF receptor Flt-1/KDR, SU5416 (semaxanib; Pharmacia), was reported to increase hematocrit in several patients participating in a clinical trial, but this was attributed to the specific cancer they had since it was not observed in other patients (Richard et al. (2002) Blood 99:3851-3853). These results demonstrates that endogenous VEGF is a regulator of erythropoiesis in a broad range of species, including mice, monkeys and humans.

VEGF Antagonists and VEGF-Specific Fusion Polypeptide Traps

[0026] The methods of the invention may be achieved with the use of a broad range of VEGF antagonists, including the soluble receptor-based fusion protein described below, an anti-VEGF antibody such as bevacizumab (AVASTIN®; Genentech), and agents capable of blocking VEGF activity for example, CDP-791 (ImClone), sorafenib (NEXAVAR®, Bayer AG), sunitnib (SUTENT®, Pfizer).

[0027] In a preferred embodiment, the VEGF antagonist is a dimeric protein capable of binding VEGF with a high affinity composed of two receptor-Fc fusion protein consisting of the principal ligand-binding portions of the human VEGFR1 and VEGFR2 receptor extracellular domains fused to the Fc portion of human IgG1. Specifically, the VEGF trap consists of Ig domain 2 from VEGFR1, which is fused to Ig domain 3 from VEGFR2, which in turn is fused to the Fc domain of IgG1 (SEQ ID NO:2).

[0028] The nucleic acid constructs encoding the fusion proteins useful in the methods of the invention are inserted into an expression vector by methods known to the art, wherein the nucleic acid molecule is operatively linked to an expression control sequence. Host-vector systems for the production of proteins comprising an expression vector introduced into a host cell suitable for expression of the protein are known in the art. The suitable host cell may be a bacterial cell such as *E. coli*, a yeast cell, such as *Pichia pastoris,* an insect cell, such as *Spodoptera frugiperda*, or a mammalian cell, such as a COS, CHO, 293, BHK or NS0 cell. In a preferred embodiment, an expression plasmid encoding the VEGF trap is transfected into CHO cells, which secrete VEGF trap into the culture medium. The resulting VEGF trap is a dimeric glycoprotein with a protein molecular weight of 97 kDa and contains ~15% glycosylation to give a total molecular weight of 115 kDa.

[0029] Since the VEGF trap binds its ligands using the binding domains of high-affinity receptors, it has a greater affinity for VEGF than do monoclonal antibodies. The VEGF trap binds VEGF-A with a K_D of approximately about 1.5 pM, PLGF1 with a K_D of approximately about 1.3 nM, and PLGF2 with a K_D of approximately about 50 pM; binding to other VEGF family members has not yet been fully characterized.

Treatment Population

[0030] Subjects undergoing cancer treatment with chemotherapeutic agents and/or radiation are known to suffer severe reduction win megakaryocyte and/or erythrocyte levels and become anemic. Subjects suffering from diseases such as AIDS, aplastic anemia and myelodysplasias are similarly known to become anemic. Generally, a subject is considered anemic if their hemoglobin levels fall below 11-13 gm/100 ml of blood (depending upon the age and sex of the patient Accordingly, a subject at risk for development of anemia or suffering from anemia is a candidate for treatment by the methods of the invention.

Combination Therapies

[0031] In numerous embodiments, a VEGF antagonist may be administered in combination with one or more additional compounds or therapies, including a second VEGF antagonist molecule. Combination therapy includes administration of a single pharmaceutical dosage formulation which contains a VEGF antagonist and one or more additional agents; as well as administration of a

VEGF antagonist and one or more additional agent(s) in its own separate pharmaceutical dosage formulation. For example, a VEGF antagonist and a cytotoxic agent, a chemotherapeutic agent or a growth inhibitory agent can be administered to the patient together in a single dosage composition such as a combined formulation, or each agent can be administered in a separate dosage formulation. Where separate dosage formulations are used, the VEGF-specific fusion protein of the invention and one or more additional agents can be administered concurrently, or at separately staggered times, i.e., sequentially.

[0032] 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. I^{131}, I^{125}, Y^{90} and Re^{186}), chemotherapeutic agents, and toxins such as enzymatically active toxins of bacterial, fungal, plant or animal origin, or fragments thereof.

[0033] A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include alkylating agents such as thiotepa and cyclosphosphamide (Cytoxan®); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethylenethiophosphaoramide and trimethylolomelamine; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; antibiotics such as aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, calicheamicin, carabicin, carminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfornithine; elliptinium acetate; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK®; razoxane; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2, 2',2"-

trichlorotriethylamine; urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxanes, e.g. paclitaxel (Taxol®, Bristol-Myers Squibb Oncology, Princeton, N.J.) and docetaxel (Taxotere®; Aventis Antony, France); chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitomycin C; mitoxantrone; vincristine; vinorelbine; navelbine; novantrone; teniposide; daunomycin; aminopterin; xeloda; ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoic acid; esperamicins; capecitabine; 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 including for example tamoxifen, raloxifene, aromatase inhibiting 4(5)-imidazoles, 4-hydroxytamoxifen, trioxifene, keoxifene, LY 117018, onapristone, and toremifene (Fareston); and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

[0034] A "growth inhibitory agent" when used herein refers to a compound or composition which inhibits growth of a cell, especially a cancer cell either *in vitro* or *in vivo*. 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.

Methods of Administration

[0035] The invention provides compositions and methods of treatment comprising a VEGF antagonist, such as a VEGF antagonist, and an anti-hypertensive agent. Various delivery systems are known and can be used to administer the composition of the invention, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the compound, receptor-mediated endocytosis (see, *e.g.*, Wu and Wu, 1987, J. Biol. Chem. 262:4429-4432), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of introduction can be enteral or parenteral and include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, intraocular, and oral routes. The compounds may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. Administration can be acute or chronic (e.g. daily, weekly, monthly, etc.) or in

combination with other agents. Pulmonary administration can also be employed, *e.g.*, by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

[0036] In another embodiment, the active agent can be delivered in a vesicle, in particular a liposome, in a controlled release system, or in a pump. In another embodiment where the active agent of the invention is a nucleic acid encoding a protein, the nucleic acid can be administered *in vivo* to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, *e.g.*, by use of a retroviral vector (see, for example, U.S. Patent No. 4,980,286), by direct injection, or by use of microparticle bombardment, or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (see *e.g.*, Joliot et al., 1991, Proc. Natl. Acad. Sci. USA 88:1864-1868), etc.

[0037] In a specific embodiment, it may be desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment; this may be achieved, for example, and not by way of limitation, by local infusion during surgery, topical application, *e.g.*, by injection, by means of a catheter, or by means of an implant, the implant being of a porous, non-porous, or gelatinous material, including membranes, such as silastic membranes, fibers, or commercial skin substitutes.

[0038] A composition useful in practicing the methods of the invention may be a liquid comprising an agent of the invention in solution, in suspension, or both. The term "solution/suspension" refers to a liquid composition where a first portion of the active agent is present in solution and a second portion of the active agent is present in particulate form, in suspension in a liquid matrix. A liquid composition also includes a gel. The liquid composition may be aqueous or in the form of an ointment.

[0039] An aqueous suspension or solution/suspension useful for practicing the methods of the invention may contain one or more polymers as suspending agents. Useful polymers include water-soluble polymers such as cellulosic polymers and water-insoluble polymers such as cross-linked carboxyl-containing polymers. An aqueous suspension or solution/suspension of the present invention is preferably viscous or muco-adhesive, or even more preferably, both viscous and mucoadhesive.

Pharmaceutical Compositions

[0040] Pharmaceutical compositions useful in the practice of the method of the invention include a therapeutically effective amount of an active agent, and a pharmaceutically acceptable carrier. The term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly, in humans. The term "carrier" refers to a diluent, adjuvant,

excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin.

[0041] In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous, subcutaneous, or intramuscular administration to human beings. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lidocaine to ease pain at the site of the injection. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[0042] The active agents of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with free amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl groups such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

[0043] The amount of the active agent of the invention that will be effective in the treatment of diabetes can be determined by standard clinical techniques based on the present description. In addition, *in vitro* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the condition, and should be decided according to the judgment of the practitioner and each subject's circumstances. However, suitable dosage ranges for intravenous administration are generally about 50-5000 micrograms of active compound per kilogram body weight. Suitable dosage ranges for intranasal administration are generally about 0.01 pg/kg body weight to 10 mg/kg body weight. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

[0044] For systemic administration, a therapeutically effective dose can be estimated initially from *in vitro* assays. For example, a dose can be formulated in animal models to achieve a circulating concentration range that includes the IC_{50} as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Initial dosages can also be estimated from *in vivo* data, e.g., animal models, using techniques that are well known in the art. One having ordinary skill in the art could readily optimize administration to humans based on animal data.

[0045] Dosage amount and interval may be adjusted individually to provide plasma levels of the compounds that are sufficient to maintain therapeutic effect. One having skill in the art will be able to optimize therapeutically effective local dosages witho ut undue experimentation.

[0046] The amount of compound administered will, of course, be dependent on the subject being treated, on the subject's weight, the severity of the affliction, the manner of administration, and the judgment of the prescribing physician. The therapy may be repeated intermittently while symptoms are detectable or even when they are not detectable. The therapy may be provided alone or in combination with other drugs.

[0047] Other features of the invention will become apparent in the course of the following descriptions of exemplary embodiments which are given for illustration of the invention and are not intended to be limiting thereof.

EXAMPLES

[0048] The following example is put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the methods and compositions of the invention, and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

Example 1. Effect of VEGF Trap Administration on Hematocrit in Mice

[0049] SCID mice (n=3-4) were treated with VEGF trap protein (SEQ ID NO:4) injected subcutaneously twice weekly (cumulative weekly doses of 5 and 50 mg/kg) or thrice iweekly (cumulative weekly dose of 75 mg/kg) over a period of 8 or 9.5 weeks, respectively. Hematocrit was measured by standard technique. The results are shown in Fig. 1. Increased hematocrit was observed in a dose-dependant manner in SCID mice treated with VEGF trap.

[0050] Mice were treated with twice weekly subcutaneous injections of 2.5 or 25 mg/kg for 4 or 8 week periods. Hematocrit levels were measured in mice sacrificed 4 and 8 weeks. The results are shown in Fig. 2. Increased hematocrit was observed in mice in both VEGF trap dosing cohorts after

4 weeks of treatment and this effect became more pronounced with extended treatment

Example 2. Effect of VEGF Trap Administration on Hematocrit in Monkeys

[0051] Twenty-four cynomolgus monkeys (3/sex/group) were administered subcutaneously 1.5, 5 or 15 mg/kg VEGF trap protein (SEQ ID NO;4) or vehicle three times a week for four weeks. Eight additional cynomolgus monkeys (2/sex/group) received either vehicle or 15 mg/kg VEGF trap protein three times a week for four weeks with an additional 28-day recovery period. An increase in hematocrit, hemoglobin and red blood cell counts was observed at all doses.

[0052] Cynomolgus monkeys (n=6) were subcutaneously injected biweekly with VEGF trap protein (SEQ ID NO:4) at 15 and 30 mg/kg. Hematocrit levels were determined at 13 weeks of treatment. Results are shown in Fig. 3.

Example 3. Effect of VEGF Trap Administration on Hematocrit in Mice

[0053] VEGF Trap was administered twice weekly via subcutaneous injection at doses ranging between 0 and 25 mg/kg for periods ranging from 10 days to 8 weeks. At the termination of the study, whole blood was obtained by cardiac puncture and sent to AniLytics (Gaithersburg, MD) for analysis including determination of hematocrit. "IgG4" refers to a version of the VEGF trap protein in which IgG4 is substituted for IgG1; "cremophor" is a vehicle for paclitaxel (Taxol™) comprising 10% cremophor, 10% ethanol in PBS; Taxol™ was given as 20 mg/kg three times per week. When mice are treated with VEGF Trap, hematocrit is increased regardless of the strain of mouse. In general, effects of VEGF trap treatment on hematocrit appear to be dependant on both duration of treatment as well as dose. When VEGF trap was combined with an agent that alone resulted in decreased hematocrit, hematocrit was found to be at approximately normal levels.

Table 1. Effect of VEGF Trap Administration on Hematocrit

Experiment	Strain/sex	Duration	Dose VEGF Trap	% Hematocrit
VGT1	CD-1/F	10 days	0	42.7 ± 1.4
			2.5 mg/kg	35.3 ± 3.7
			25 mg/kg	37.8
	CD-1/M	10 days	0	38.4 ± 3.2
			2.5 mg/kg	39.5 ± 3.6
			25 mg/kg	48.3 ± 3.6
VGT2	SCID/F	3 weeks	0	40.1 ± 1.3
			25 mg/kg	49 ± 2.9
	SCID/M	3 weeks	0	39.5 ± 2.8
			25 mg/kg	56.0 ± 0.6
VGT3	SCID/M (15 weeks)	4 weeks	0	38.3 ± 0.6
			2.5 mg/kg	42.8 ± 4.1
			25 mg/kg	48.2 ± 4.8

	SCID/M (7 weeks)	4 weeks	0	43.1 ± 0.6
			2.5 mg/kg	46.3 ± 3.7
			25 mg/kg	53.0 ± 1.5
	SCID/M (15 weeks)	8 weeks	0	39
			2.5 mg/kg	57.7 ± 1.8
			25 mg/kg	62.2 ± 3.3
	SCID/M (7 weeks)	8 weeks	0	40.6 ± 1.1
			2.5 mg/kg	59.4 ± 2.0
			25 mg/kg	64.0 ± 3.2
VGT4	SCID/M	8 weeks	0	40.5 ± 1.6
			5 mg/kg	55.2 ± 1.7
			5 mg/kg IgG4	53.8 ± 1.7
VGT5	Nod/SCID/M	4 weeks	0	33.7 ± 2.5
			2.5 mg/kg	37.4 ± 6.8
			25 mg/kg	41.6 ± 1.1
		6 weeks	0	35.3 ± 0.7
			2.5 mg/kg	41.1 ± 2.3
			25 mg/kg	46.0 ± 4.7
VGT6	SCID	8 weeks	0	41.4 ± 3.4
			5 mg/kg	58.4 ± 3.2
			25 mg/kg	54.1 ± 0.6
VGT8	SCID	4 weeks	0	40.9 ± 2.3
			25 mg/kg	51.4 ± 0.6
	C57B1/6	4 weeks	0	43.2 ± 0.8
			25 mg/kg	59.5 ± 1.4
VTC1	Nude	4 weeks	Taxol™	36.2 ± 0
			Cremophor	41.5 ± 1.7
			0	42.4 ± 0.4
			1 mg/kg + Taxol™	42.3 ± 1.3
			5 mg/kg + Taxol™	51.2
			25 mg/kg + Taxol™	45.6
			25 mg/kg +cremophor™	46.1 ± 6.7
			25 mg/kg	49.5 ± 1.8

Example 4. Effect of VEGF Trap on Hematocrit in Human Patients

[0054] A clinical trial was conducted with VEGF trap protein (SEQ ID NO:4) administered subcutaneously into subjects with incurable relapased or refractory solid tumors, other than squamous-cell lung cancer. Subjects were treated with VEGF trap protein at 25, 50, 100, 200, 400, and 800 μg/kg weekly, and 800 μg/kg twice weekly. Beginning in the fifth week of study, subjects received a series of 6 weekly (or twice weekly) injections at the assigned dose level.

[0055] A dose-related increase in hematocrit values was observed between baseline and visit 16. Hematocrit values changed by +3.8%, +2.7%, -0.2%, +1.6%, +0.6% and +0.1% in the 800 μg/kg twice weekly and weekly groups, and 400, 200, 100, 50, and 25 μg/kg dose level cohorts, respectively. Similar observations were seen for hemoglobin.

[0056] Dose level cohorts were combined into 4 dose groups to facilitate the use statistical tools to examine the trends in hemoglobin and hematocrit by creating groups for analysis with approximately equal numbers of subjects. Combined dose group 1 included subjects treated at the 25, 50, and 100 μg/kg (combined n=13); combined dose group 2 included subjects treated at the 200 and 400 μg/kg dose levels (combined n-10); combined dose group 3 included subjects treated at the 800 μg/kg once weekly dose level (n-7), and dose group 4 included subjects treated at the 800 μg/kg twice weekly dose level (n=8). While mean hematocrit did not increase in the cohort of patients in the lowest dose group (25, 50 and 100 mg), in each of the other three groups an increase in hematocrit was apparent after 4 weeks of treatment. After 10 weeks of treatment this effect was most pronounced in the two highest dose groups (800 mg once or twice weekly).

We claim,

1. Use of an agent capable of blocking, inhibiting, or ameliorating vascular endothelial growth factor (VEGF)-mediated activity in the preparation of a medicament for: (a) the treatment of an anemic disorder in a mammal; (b) preventing anemia expected to result from cancer treatment in a subject at risk thereof; (c) increasing hematocrit level in a subject in need thereof; or (d) increasing erythropoietin levels or stimulating erythropoiesis in a subject in need thereof.

2. The use of claim 1, part (a), wherein the anemic disorder is characterized by a reduced hematocrit level.

3. The use of claim 1, part (a), or claim 2, wherein the anemic disorder is associated with chronic renal failure, cancer, cancer chemotherapy, radiation treatment, antiviral therapy, nutritional iron-deficiency, blood-loss, or hemolysis.

4. The use of claim 3, wherein the anemic disorder results from treatment with chemotherapy or radiation.

5. The use of claim 1, part (b), wherein cancer treatment is treatment with radiation and/or chemotherapy.

6. The use of claim 1, part (c), wherein the hematocrit level in a subject in need thereof is less than about 33% of total blood volume and administration of the fusion polypeptide is in an amount sufficient to increase the hematocrit level above 33% of total blood volume.

7. The use of any one of the preceding claims , wherein the agent is VEGFR1R2-FcΔC1(a) (SEQ ID NO:4).

8. The use of any one of the preceding claims, wherein administration is subcutaneous or intravenous.

9. The use of any one of the preceding claims wherein administration is in a single dose or multiple doses.

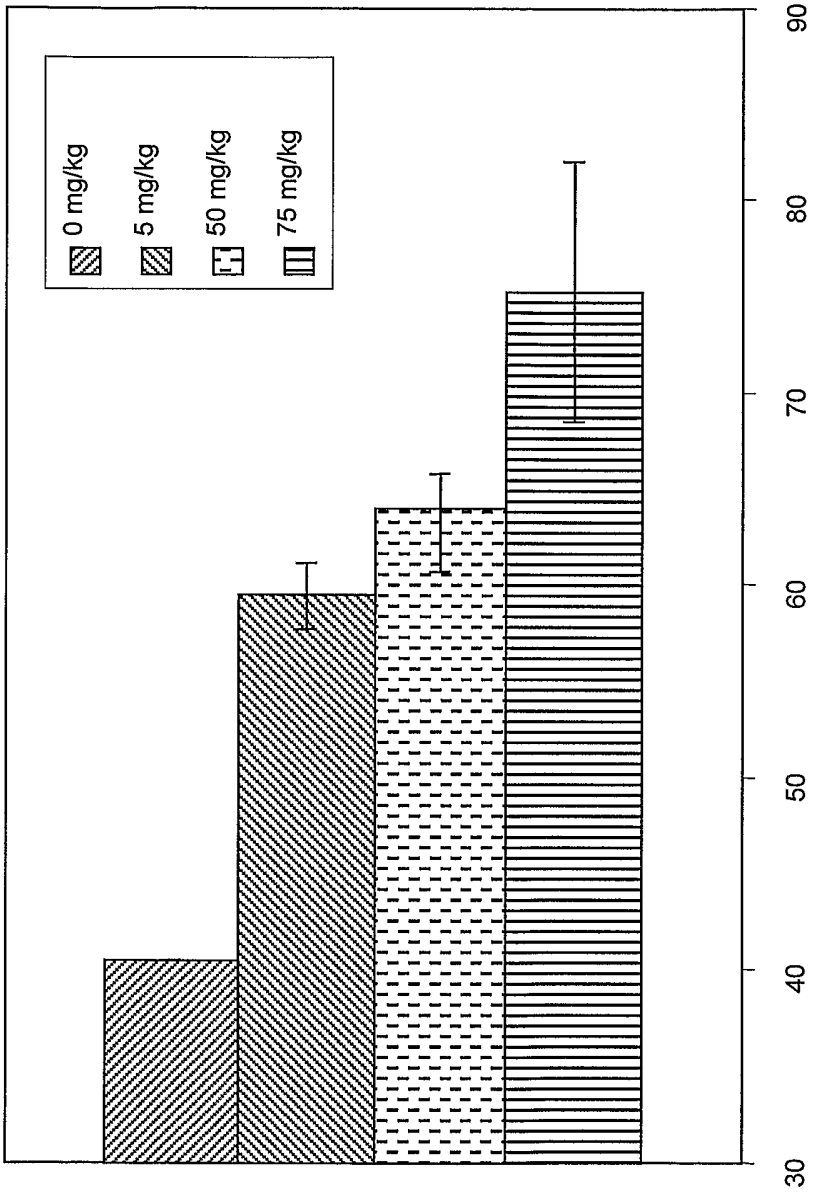

Fig. 1

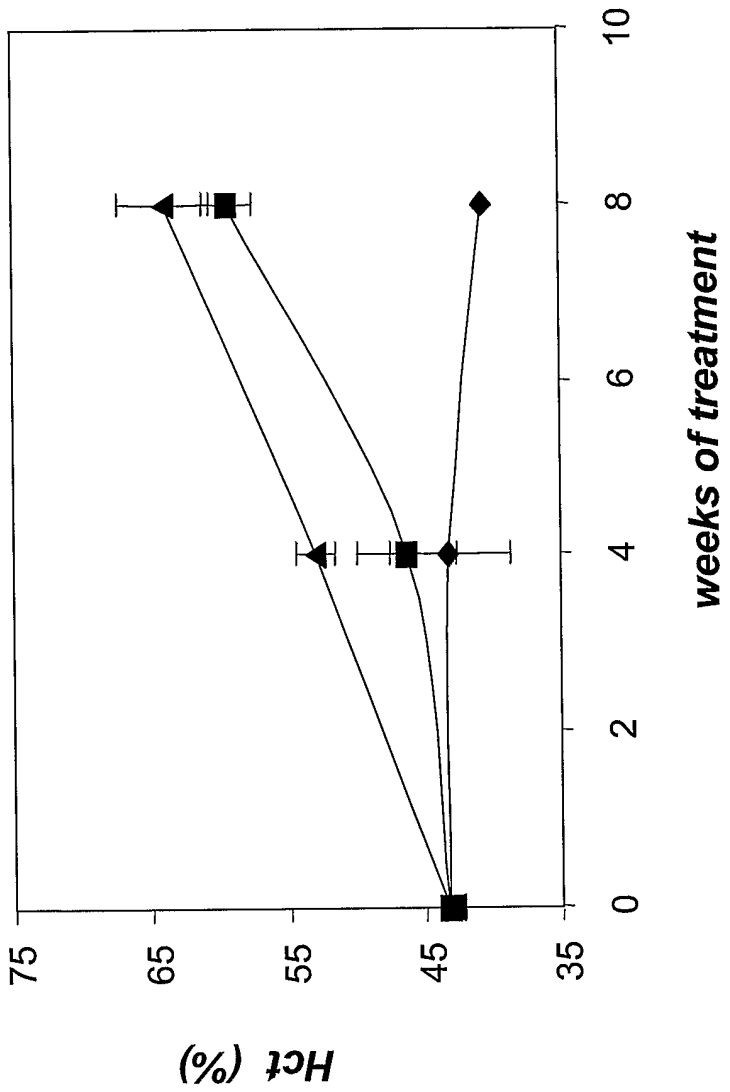

Fig. 2

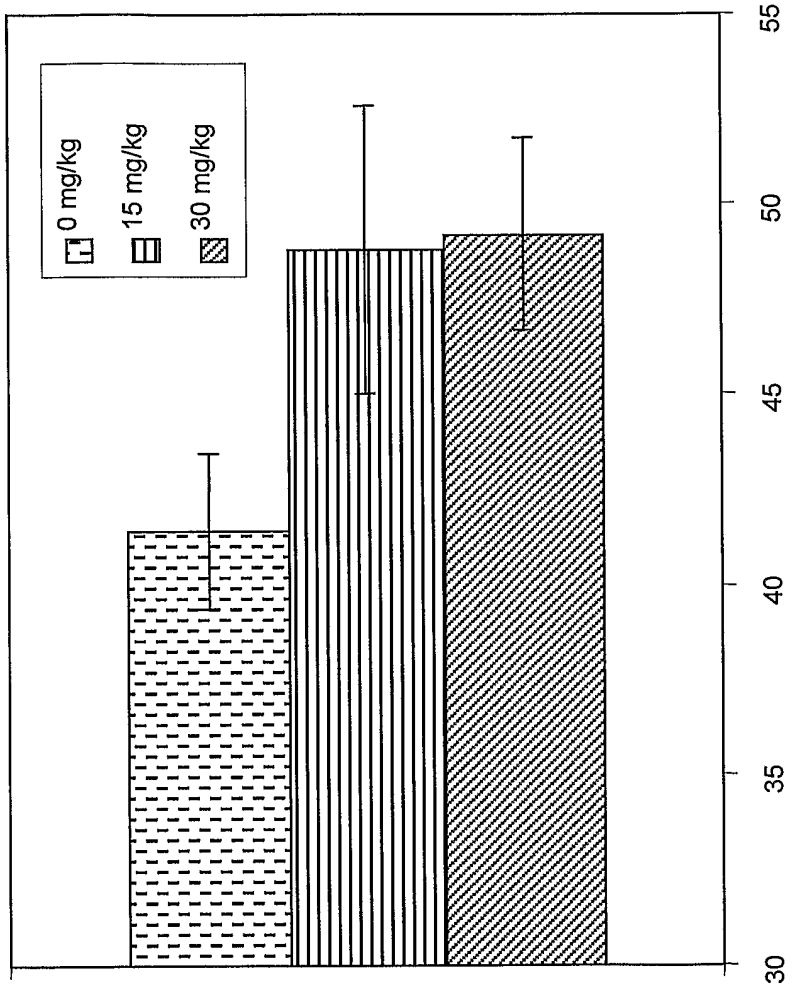

Fig. 3

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2006/008620

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K38/17 A61P7/06

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

B. FIELDS SEARCHED

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

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TAM BETTY Y Y ET AL: "Hypoxia-independent regulation of hepatic erythropoietin production by vascular endothelial growth factor." BLOOD, vol. 104, no. 11, Part 1, November 2004 (2004-11), page 595A, XP002391704 & 46TH ANNUAL MEETING OF THE AMERICAN-SOCIETY-OF-HEMATOLOGY; SAN DIEGO, CA, USA; DECEMBER 04 -07, 2004 ISSN: 0006-4971 abstract	1-9

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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

"E" earlier document 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

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 July 2006	11/08/2006

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Lechner, O

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2006/008620

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	RICHARD STEPHANE ET AL: "Paradoxical secondary polycythemia in von Hippel-Lindau patients treated with anti-vascular endothelial growth factor receptor therapy" BLOOD, vol. 99, no. 10, 15 May 2002 (2002-05-15), pages 3851-3853, XP002391717 ISSN: 0006-4971 cited in the application the whole document	1-3,8,9
X	WO 2005/016369 A (REGENERON PHARMACEUTICALS, INC; HOLASH, JOCELYN; YANCOPOULOS, GEORGE;) 24 February 2005 (2005-02-24)	1,7-9
A	the whole document	1-9
A	KONNER J ET AL: "USE OF SOLUBLE RECOMBINANT DECOY RECEPTOR VASCULAR ENDOTHELIAL GROWTH FACTOR TRAP (VEGF TRAP) TO INHIBIT VASCULAR ENDOTHELIAL GROWTH FACTOR ACTIVITY" CLINICAL COLORECTAL CANCER, XX, US, vol. 4, no. SUPPL 2, October 2004 (2004-10), pages S81-S85, XP009050556 ISSN: 1533-0028 the whole document	1-9

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.2

Claims 1-6, 8, 9 are unclear (Art. 6, PCT) as far as relating to subject matter defined in terms of the result to be achieved, i.e. agent capable of blocking, inhibiting or ameliorating VEGF-mediated activity, i.e. in terms of functional features. Although these functional features can be tested with the methods disclosed in the present application, the skilled person cannot understand the definition of the claimed subject-matter, because the claimed compounds are not limited by any structural definition and therefore no limits to the number of compounds to be tested for the modulating activity exist. The claim does not provide any criterion to discriminate between known and novel compounds. Thus, the lack of clarity is to such an extent that a meaningful search over the entire scope of said claims cannot be carried out. Consequently, the search has been carried out for those parts of the claims which appear to be clear in view of the description, namely those parts relating to molecules encoded by SeqID 2 and 4 (c.f. p 2), SU5416 (p 5, 25), anti-VEGF Ab, CDP-791, sorafenib, sunitnib (26-27).

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.5), should the problems which led to the Article 17(2) declaration be overcome.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2006/008620

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [ ] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. [X] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2006/008620

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2005016369 A	24-02-2005	AU 2004264891 A1	24-02-2005
		CA 2534197 A1	24-02-2005
		EP 1653992 A1	10-05-2006