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- (54) **Title:** ANTI-HUMAN VEGF ANTIBODIES WITH UNUSUALLY STRONG BINDING AFFINITY TO HUMAN VEGF-A AND CROSS REACTIVITY TO HUMAN VEGF-B

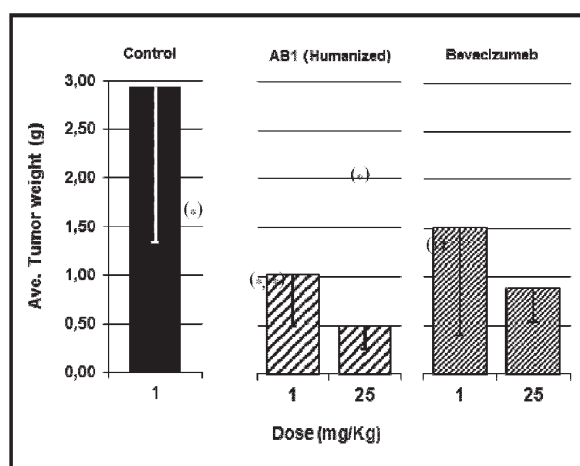


FIG. 5

(57) **Abstract:** Murine and humanized anti-human VEGF antibodies and uses are disclosed. The anti-human VEGF antibodies of the invention have higher binding affinity for human VEGF-A, are stronger inhibitors of the VEGF-A induced proliferation of endothelial cells in culture as compared with anti-human VEGF antibodies in the art. Moreover, these antibodies cross react with human VEGF-B. The antibodies of the invention inhibit tumor growth in vivo in greater extent than Bevacizumab (AvastinTM) when administered at the same dosage



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**ANTI-HUMAN VEGF ANTIBODIES WITH UNUSUALLY STRONG BINDING
AFFINITY TO HUMAN VEGF-A AND CROSS REACTIVITY TO HUMAN VEGF-
B**

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BACKGROUND OF THE INVENTION

Field of the invention

This invention relates generally to anti-human VEGF antibodies with unusually high binding affinity for human VEGF-A and which also cross react with human VEGF-B and particularly to chimeric and humanized versions these antibodies.

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Description of related art

Angiogenesis is a physiological process that involves the growth of new blood vessels from pre-existing ones. This process is vital for growth and development as well as for wound healing. However, it is also fundamental for malignant tumor development and for other non-tumor associated pathologies such as proliferative retinopathies, rheumatoid arthritis and psoriasis (Folkman J. 1971; Praidou A et al.2010; Canavese M et al. 2010). Vascular endothelial growth factor (VEGF) is a protein secreted by stromal and tumor cells and in a minor proportion by a variety of other cells that plays a key role in stimulation of Angiogenesis (Ferrara N et al. 2004). Over-expression of VEGF can contribute to angiogenesis related diseases. For example, solid cancers cannot grow beyond a limited size without an adequate blood supply and cancers that express VEGF are more capable of growing and metastasizing (Ferrara N. 2005). Taking into account these considerations, anti-angiogenic therapies were developed using anti-VEGF monoclonal antibodies such as Bevacizumab (Avastin) for cancer treatment (Ferrara N et al. 2005) and Ranibizumab (Lucentis) for retinopathy (Ciulla TA et al. 2009). Therapies based in the use of Bevacizumab and Ranibizumab have been extensively studied and applied during the last few years (Van Meter ME et al. 2010; Ciulla TA et al. 2009). Bevacizumab was approved by the U.S. Food and Drug Administration (FDA) for metastatic renal carcinoma, glioblastoma, metastatic colon cancer and non-small cell lung cancer in combination with standard chemotherapy and for use in metastatic breast cancer. However, in the US, a FDA panel of experts has now said they do not see enough of a benefit from Avastin in advanced breast cancer to justify its serious risks. Several clinical trials are also currently in progress in non-metastatic breast cancer, renal cell carcinoma, glioblastoma multiforme,

ovarian cancer, castrate-resistant prostate cancer, non-metastatic unresectable liver cancer and metastatic or unresectable locally advanced pancreatic cancer. Bevacizumab is also used without FDA approval, but on the basis of clinical studies, for treatment of macular degeneration, an eye disease also characterized by proliferation of blood vessels in the retina. Ranibizumab is a Fab antibody fragment derived from the same murine antibody as Bevacizumab and has been approved by the FDA to treat the wet age-related macular degeneration.

In spite of the benefits resulting of the clinical use of the currently approved anti-VEGF-A antibodies, numerous problems remain to be solved regarding to therapeutic effects, side effects, resistance to treatment and cost of treatments. Therefore, development of new more effective anti-human VEGF antibodies is necessary. We have now developed new anti-human VEGF antibodies witch surprisingly have unusually high binding affinity for the human VEGF-A target and cross-reactivity with human VEGF-B. Even though VEGF-B is strongly related to VEGF-A according to its amino acid sequence, rather than an angiogenic function has a potent survival/antiapoptotic effect required for blood vessel survival (Li X et al.2009). Therefore, VEGF-B is very important to maintain integrity of the blood vessels which support tumor growth and interference in its action should contribute to a more complete anti-tumor therapy.

SUMMARY OF THE INVENTION

-This application describes anti-human VEGF antibodies with unusually strong binding affinity for human VEGF-A and cross reactivity with human VEGF-B, which are stronger inhibitors of the VEGF induced proliferation of endothelial cells in culture as compared with anti-human VEGF antibodies in the art.

-The murine anti-human VEGF antibodies disclosed herein binds human VEGF-A with a K_D value of no more than 5×10^{-12} M and one humanized version of anti-human VEGF disclose herein binds human VEGF-A with a K_D value of no more than 1.0×10^{-10} M. Furthermore, the mouse anti-human VEGF antibodies disclosed herein have an IC_{50} value of about 11 ng/ml for inhibiting VEGF-A induced proliferation of HUVEC endothelial cells *in vitro* and its humanized version have an IC_{50} value of about 19 ng/ml for inhibiting VEGF-A induced proliferation of HUVEC endothelial cells *in vitro*. The anti-human VEGF antibodies of the present invention bind to three regions of human VEGF-A₁₆₅ (SEQ ID NO:1) encompassing amino acids residues 16 to 25, 45 to 54 and 79 to 92 of the

human VEGF-A₁₆₅ (SEQ ID NO:1). All of these regions are involved in the interaction between VEGF-A and its receptor (VEGFR). In particular amino-acids 16 to 25 of the VEGF-A₁₆₅ are involved in an important contact region (Shalini L et al.2010).Regarding to this, it has been pointed out that monoclonal antibodies against this region may be very difficult to obtain by the traditional method used to obtain monoclonal antibodies against human VEGF (Jin Kim K et al. 1993) because of the high homology between human and mouse VEGF in the segment comprising amino-acids 16 to 25 (Fuh G et al.2006). However, a modification in the scheme of immunization disclosed in this invention surprisingly resulted in the obtainment of monoclonal antibodies against this region using the classical method (Kohler et al.1975). These monoclonal antibodies have unusual high affinity to the VEGF-A molecule and also present cross reactivity with human VEGF-B (SEQ ID NO: 2).

- Within the scope of this invention is the use of the humanized version of the antibodies here disclosed for treating VEGF –related angiogenic diseases, including cancer, age-related macular degeneration, rheumatoid arthritis and diabetic retinopathy, by administering to a subject in need of the treatment a therapeutically effective amount of the antibody.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig 1 depicts inhibition of VEGF-induced mitogenesis of a culture of HUVEC cells by the mouse mMCA-AB1-anti-VEGF-A monoclonal antibody of this invention and inhibition of VEGF-induced mitogenesis of a culture of HUVEC cells by the anti-VEGF monoclonal antibody Bevacizumab (AvastinTM). Experimental procedures were as described in EXAMPLE 5. Each point corresponds to the average and standard deviation of 6 repetitions. IC₅₀ values were calculated with the *GraphPad Prism 5* program.

Fig 2 depicts inhibition of VEGF-induced mitogenesis of a culture of HUVEC cells by the humanized version of the mouse mMCA-AB1-anti-VEGF-A monoclonal antibody of this invention and inhibition of VEGF-induced mitogenesis by the anti-VEGF monoclonal antibody Bevacizumab (AvastinTM). Experimental procedures are described in EXAMPLE 5. Each point corresponds to the average and standard deviation of 6 repetitions. IC₅₀ values were calculated with the *GraphPad Prism 5* program.

Fig 3 depicts inhibition of IIB-Mel-J tumor growth in nude mice after treatment with the mouse mMCA-AB1-anti-VEGF-A monoclonal antibody of this invention and with

the humanized version of the mouse mMcA-AB1-anti-VEGF-A monoclonal antibody of this invention at the following doses: 1 µg/mouse or 25 µg/mouse, twice a week for 5 weeks. After this animals were killed and tumors excised and weighed. (*): statistically significance according to the non-parametric Kruskal Wallis test.

5 Fig 4 depicts tumor images illustrating the inhibition of IIB-Mel-J tumor growth in nude mice as a function of the dose of the humanized version of the mouse mMcA-AB1-anti-VEGF-A monoclonal antibody of this invention or the anti-VEGF monoclonal antibody Bevacizumab (AvastinTM).

Fig 5 depicts inhibition of IIB-Mel-J tumor growth in nude mice after treatment with the humanized version of the mouse mMcA-AB1-anti-VEGF-A monoclonal antibody of this invention or Bevacizumab (AvastinTM) at the following doses: 1 µg/mouse or 25 µg/mouse, twice a week for 5 weeks. After this animals were killed and tumors excised and weighed. (*): statistically significance to the control according to the non-parametric Kruskal Wallis test. (**): statistically significance to the Bevacizumab treatment according to the non-parametric Kruskal Wallis test.

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DETAILED DESCRIPTION OF THE INVENTION

The following description of the invention merely intends illustrate several embodiments of the invention. However, by not means these discussed embodiments limit the scope of the invention since many changes and modifications will be apparent to one skilled in the art without departing from the scope of the invention.

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ABBREVIATIONS

The following abbreviations are used throughout the present invention: **VEGF**= vascular endothelial growth factor; **VEGF-A**= vascular endothelial growth factor variant A; **VEGF-B**= vascular endothelial growth factor variant B; **VEGFR**= vascular endothelial growth factor receptor; **HUVEC** = Human Umbilical Vein Endothelial Cells; **KD** = dissociation constant of a particular antibody-antigen interaction; **BSA** = Bovine serum albumin.

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DEFINITIONS

The term “**human VEGF**” as used herein refers to the 165-amino acid vascular cell growth factor (**VEGF-A₁₆₅**) (SEQ ID NO:1), and described related vascular endothelial

growth factors (Leung DW et al 1989, Houck, KA et al 1991) and allelic and processed forms of those growth factors as described (Ferrara N. 1999).

The term “**human VEGF-B**” as used herein refers to the 186-amino acid vascular cells growth factor variant (**VEGF-B₁₈₆**) (SEQ ID NO:2) which has a critical role in blood vessel survival during pathological conditions (Zhang F et al. 2009).

The term “**antibody**” as used herein includes any of the following molecular species: monoclonal antibody, polyclonal antibody, multispecific antibody, or bispecific antibody. A normal antibody comprises two heavy chains and two light chains. Each heavy chain consist of a variable region and a constant region. Each heavy chain has two regions, the constant region and the variable region. Each light chain has also a constant region and a variable region. The variable regions of the light and heavy chains are responsible for antigen binding. The variable region in both chains generally contains three variable loops named complementary determining regions (CDR_S). The three CDR_S are inserted between flanking segments named framework region (FR_S). The FR_S is significantly more conserved than CDR_S.

Antibodies are grouped into different isotypes based on the structure of their heavy chain. There are five major isotypes of antibodies named IgA, IgD, IgE, IgG, and IgM.

The term “antigen-binding fragment” as used herein refers to an antibody fragment the binds to a given antigen but do not comprise a complete antibody. Some, but not all, well known in the art examples of antigen-binding fragments are: Fab, F(ab')₂, Fv, and scFv. In certain embodiments, an antigen-binding fragment may comprise one or more CDRs from a specific antibody combined with FRs of one or more human antibodies.

“Fab” refers to an antibody fragment that binds to antigens and it is composed of one constant and one variable domain of each of the heavy and the light chain. In an experimental setting, Fab fragments can be generated by digestion of an antibody with the enzyme papain.

“F(ab')₂” refers to an antibody fragment that can be obtained by digestion of an antibody with the enzyme pepsin that cleaves below the hinge antibody region.

“Fv” refers to the smallest fragment of an antibody to bear the complete antigen binding site and that consist of the variable region of the light chain bound to the variable region of the heavy chain of the same antibody.

“scFv” refers to an engineering molecule consisting of two Fv fragments connected by a peptide linker (Huston, J. S. et al.1988).

The term “epitope” as used herein refers to a special region of an antigen where an antibody binds.

The term “Cancer” as used herein refers to a medical condition involving uncontrollably cell division that results in formation of malignant tumors and metastasis.

5 The term “angiogenesis” as used herein refers to a physiological process involving the growth of new blood vessels from pre-existing vessels.

The phrase “disease associated with excessive angiogenesis” as used herein refers to a medical condition caused by an abnormal increase in angiogenesis.

10 ***Therapeutic Monoclonal Antibodies with High Affinity for its Target Antigen***

Monoclonal antibodies affinity for its target antigen is a critical factor to be taken into account for therapeutic performance (Harms BD et al 2012; Lippow MS et al 2007; Zhou Y, et al 2012). Antibodies of higher affinity may be able to be used at lower doses to achieve the desired clinical effects. Lower dosing may allow for more convenient routes of administration and decreased injection volumes, which would translate into lower cost of goods.

Antibodies reported to be of high affinity are generally in the nanomolar range (Griffiths AD, et al 1994; de Haard HJ, et al 1999) and occasionally in the sub-nanomolar range (Vaughan TJ et al 1996). In particular, the K_D of the well known anti-VEGF therapeutic monoclonal antibody AVASTINTM (bevacisumab) is in the nanomolar range (Presta, L. G., et al 1997).

There are few reports describing monoclonal antibodies with a K_D in the picomolar range and all of them were obtained using non-conventional techniques (Yang W et al 1995; Rathanaswami P et al 2005; Zhao Q et al 2011; Pichinuk E et al 2012).

25 The present invention provides anti-VEGF monoclonal antibodies which bind human VEGF-A₁₆₅ (SEQ ID NO: 1) with a K_D value of about 5.0 pM in its mouse version, and a K_D of about 100 pM in one humanized version.

- Both, the mouse anti-VEGF monoclonal antibody and the humanized version of this invention also have cross-reactivity with human VEGF-B (SEQ ID NO: 2).
- 30 - The murine version of the monoclonal antibodies of this invention neutralizes VEGF-A in the HUVEC proliferation assay with an IC₅₀ of about 11 ng/ml (five times lower than the IC₅₀ of bevacizumab in the same assay), and the humanized

version of the antibodies of this invention in a concentration of about 19 ng/ml (three times lower than the IC₅₀ of bevacizumab in the same assay).

- Both, the mouse anti-VEGF monoclonal antibody and the humanized version of this invention have been characterized as possessing superior anti-tumor activity in vivo than Bevacizumab. This is not at all an obvious result since numerous factors determine the anti-tumor activity in vivo (Rudnick SI et al 2009; Beckman RA et al 2007; Covell DG et al 1986; Thurber GM et al 2007; Jain RK et al 1990; Liang W et al 2006). Therefore, the discovery of an antibody with very high affinity and simultaneously very high biological activity in vivo such as the one disclosed herein is highly unpredictable.

Disclosed herein are the parental mice antibody named mMCA-AB1-anti-VEGF-A (mouse monoclonal antibody A-AB1-anti-VEGF-A), the cell line (hybridoma) that produces it named mMCA-AB1-anti-VEGF-A clone, a humanized version of mMCA-AB1-anti-VEGF-A named hMCA-AB1-VEGF-A (humanized monoclonal antibody A-AB1-anti-VEGF-A) and an engineered mini-version of mMCA-AB1-anti-VEGF-A. As discussed below, the parental antibody mMCA-AB1-anti-VEGF-A was obtained using in general the Kohler's method (Kohler G et al 1975) but employing an unusual immunization scheme as described in EXAMPLE 1. In order to select cell clones producing anti-humanVEGF-A antibodies of high biological efficiency, the HUVEC proliferation assay was used (for technical description of this assay see EXAMPLE 5). The cell clone (hybridoma) producing the most active antibody was the above mention mMCA-AB1-anti-VEGF-A clone. To our surprise, the monoclonal antibody produced by this cell clone has a very unusual high affinity (K_D in the pM range) for the human VEGF-A₁₆₅ (SEQ ID NO: 1) angiogenic factor as measured using the biacore T100 sensor (for technical description of this assay see EXAMPLE 2). Amino acid sequences of the heavy and light variable chains of mMCA-AB1-anti-VEGF-A are:

Variable Heavy Chain:

QVKLLESGPELKKPGETVKISCKASGYTFTNFGMNWVKQAPGKGLKWMGWINTN
TGEPTYVDDFKGRFAFSLETSASSAYLQISNLNEDTATYFCARYYGSTSVWYFDV

WGAGTTVTVSS (SEQ ID NO: 3)

Variable Light Chain:

ELVMTQTPSSLSASLGDRVTITCRASQDIFNYLNWYQQKPDGPIKLLIYYSSRLHSG
VPSRFSGSGSGTDYSLTISNLDREDIATYFCQQGFTLPWTFGGGGTKLEIKR (SEQ ID
NO: 4)

CDR regions are underlined

- 5 (For technical description of sequencing see EXAMPLE 6)

Epitope analysis indicated that mMcA-AB1-anti-VEGF-A bind the following three segments of the VEGF-A₁₆₅ (SEQ ID NO: 1) amino acid sequence:

1: HHEVVKFMDVYQRSYCH (aa 11 to 27 of VEGF-A₁₆₅)

2: YIFKPSCVPLMR (aa 44 to 56 of VEGF-A₁₆₅)

- 10 3: QIMRIKPHQGQHIG (aa 79 to 92 of VEGF-A₁₆₅)

(For technical description of epitope mapping see EXAMPLE 3)

- Underlined are amino acids described as critical for binding of VEGF-A to its receptor VEGFR-2 (Kiba A et al, 2003). According to this, mMcA-AB1-anti-VEGF-A is the only described monoclonal antibody able to block all the three points of close interaction between VEGF-A and its receptor VEGFR-2. This may explain at least partially the high efficiency of mMcA-AB1-anti-VEGF-A to inhibit VEGF-A activity in biological assays as compared with anti-VEGF antibodies known in the art. For example, mMcA-AB1-anti-VEGF-A exhibited the ability to inhibit HUVEC proliferation to a greater extent than Bevacizumab (see EXAMPLE 5). Also, in inhibition of tumor growth the antibodies of this invention demonstrated to be more effective at the assayed doses than Bevacizumab (see EXAMPLE 10). It is worth mention that all the assayed antibodies lack significant neutralizing activity to mouse VEGF (see EXAMPLE 1) a fact that renders comparison between its anti-tumor activities meaningful. Other anti-VEGF antibodies having neutralizing activity against mouse-VEGF have been compared, regarding to its efficiency to inhibit human tumor growth in nude mice, with Bevacizumab which do not cross-react with mouse-VEGF-A (see US patent 8,101,177 and US patent 8,034,905). Therefore, these studies are not adequate to predict the relative efficiency of these antibodies as compared with Bevacizumab in humans. Regarding to this, an illustrative discussion about the contribution of the stromal mouse VEGF to growth of human tumor xenografts can be found in Liang et al (Liang, W.C., et al., "Cross-Species Vascular Endothelial Growth Factor (VEGF)-Blocking Antibodies Completely Inhibit the Growth of Human Tumor Xenografts and Measure the Contribution of Stromal VEGF," J. Biol. Chem. 281(2):951-961 (2006)).

As discussed above, this good performance of mMCA-AB1-anti-VEGF-A in the anti-tumor assay in vivo was not at all obvious because high affinity (or even high inhibition of the activity of VEGF-A in the in vitro UVEC assay) do not necessary correlate with a high increase in the anti-tumor activity in vivo where many other factors may influence activity.

As described below (EXAMPLE 7), a superhumanized version of the parental antibody mMCA-AB1-anti-VEGF-A was obtained. For this, the framework regions between CDRs of mMCA-AB1-anti-VEGF-A were replaced for the most homologous framework regions obtained from IgG genes of human germinal cell lines (Tan P et al. 2002). This methodology introduces minimal mouse amino acid residues in order to avoid immunological reactivity during treatment of humans.

(For technical description of the superhumanization processes see EXAMPLE 7).

The sequences of the Variable Heavy and Variable Light chains of the superhumanized version of mMCA-AB1-anti-VEGF-A, named hMcA-AB1-anti-VEGF-A are:

Variable Heavy:

QVQLVQSGSELKKPGASVKVSCKASGYTFTNFGMNWVRQAPGQGLEWMGWINT
NTGEPTYVDDFKGRFVSLDTSVSTAYLQISSLKAEDTAVYYCARYYGSTSVWYF
DYWGRGTLVTVSS (SEQ ID NO: 5)

Variable Light:

DIQMTQSPSSLSASVGDRVTITCRASQDIFNYLNWYQQKPGKAPKLLIYYSSRLHS
GVPSRFSSGSGTDFFTISSLQPEDIATYYCQQGFTLPWTFGQGTKVEIKR (SEQ
ID NO: 6)

CDR regions are underlined

As its parent antibody, hMcA-AB1-anti-VEGF-A exhibited the ability to inhibit HUVEC proliferation to a greater extent than Bevacizumab (see EXAMPLE 9). Also, as its parent antibody, hMcA-AB1-anti-VEGF-A exhibited the ability to inhibit tumor-growth in vivo to a greater extent than Bevacizumab (see EXAMPLE 10).

Provided herein in certain embodiments are antibodies and antigen binding fragments that comprise the CDR sequences (SEQ ID NO: 7-12) of mMCA-AB1-anti-VEGF-A as set forth in SEQ ID NO: 5-6.

In certain embodiments, the antibodies provided herein bind VEGF-A₁₆₅ (SEQ ID NO:1) with greater affinity than that of Bevacizumab for VEGF-A₁₆₅. For example, in

certain embodiments, the antibodies provided herein bind VEGF-A₁₆₅ with a K_D of about 100 pM and in other embodiments with a K_D of about 5 pM.

The antibodies provided herein have been found to inhibit HUVEC proliferation with greater efficiency than Bevacizumab. For example in certain embodiments, the antibodies herein provided inhibit HUVEC proliferation with an ID_{50} of about 19 ng/ml and in another embodiments with an ID_{50} of about 11 ng/ml. Therefore, the antibodies provided herein may be used to treat various medical conditions associated with increased angiogenesis. For example, the antibodies may be used to treat cancer by inhibiting the proliferation of blood vessels that irrigate a tumor and therefore inhibiting the tumor growth. The anti-tumor efficiency of the antibodies herein provided has been confirmed in vivo (Example 10).

In certain embodiments, the antibodies provided herein inhibit tumor growth at a greater level than Bevacizumab. In in vivo experiments using a xenotransplanted murine tumor model, mMCA-AB1-anti-VEGF-A and hMCA-AB1-anti-VEGF-A inhibited tumor growth with more efficiency than Bevacizumab. Therefore, in certain embodiments, the antibodies provided herein may be used at similar dosage that Bevacizumab to achieve a better antitumor effect or at lesser dosage to avoid secondary undesirable side effects with similar effectivity.

The antibodies disclosed herein may be used in the treatment of medical conditions caused by excessive angiogenesis caused by excessive expression of VEGF. Examples of these conditions include wet AMD and diabetic retinopathy as ocular diseases and several tumoral diseases (Crawford TN et al, 2009; Albin A et al, 2012).

Cancer conditions that may be treated with the antibodies disclosed herein include but are not limited to carcinoma, blastoma, sarcoma, germ cell tumor, or hematological or lymphoid malignancy such as leukemia, lymphoma, or multiple myeloma. More specifically, cancerous conditions and tumor types that may be treated using the antibodies disclosed herein include but are not limited to squamous cell cancer, lung cancer (e.g., small cell lung cancer, non-small cell lung cancer (NSCLC), adenocarcinoma of the lung, or squamous cell carcinoma of the lung), cancer of the peritoneum, liver cancer (e.g., hepatocellular carcinoma/hepatoma), gastric or stomach cancer (e.g., gastrointestinal cancer), pancreatic cancer, brain tumor (e.g., glioblastoma/glioblastoma multiforme (GBM), non-glioblastoma brain tumor, or meningioma), glioma (e.g., ependymoma, astrocytoma, anaplastic astrocytoma, oligodendroglioma, or mixed glioma such as

oligoastrocytoma), cervical cancer, ovarian cancer, liver cancer (e.g., hepatoblastoma, hepatocellular carcinoma/hepatoma, or hepatic carcinoma), bladder cancer (e.g., urothelial cancer), breast cancer, colon cancer, colorectal cancer, rectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer (e.g., rhabdoid tumor of the kidney), prostate cancer, vulval cancer, penile cancer, anal cancer (e.g., anal squamous cell carcinoma), thyroid cancer, head and neck cancer (e.g., nasopharyngeal cancer), skin cancer (e.g., melanoma or squamous cell carcinoma), osteosarcoma, Ewing's sarcoma, chondrosarcoma, soft tissue sarcoma (e.g., rhabdomyosarcoma, fibrosarcoma, Kaposi's sarcoma), carcinoid cancer, eye cancer (e.g., retinoblastoma), mesothelioma, lymphocytic/lymphoblastic leukemia (e.g., acute lymphocytic/lymphoblastic leukemia (ALL) of both T-cell lineage and B-cell precursor lineage, chronic lymphoblastic/lymphocytic leukemia (CLL), acute myelogenous/myeloblastic leukemia (AML), including mast cell leukemia, chronic myelogenous/myelocytic/myeloblastic leukemia (CML), hairy cell leukemia (HCL), Hodgkin's disease, non-Hodgkin's lymphoma, chronic myelomonocytic leukemia (CMML), follicular lymphoma (FL), diffuse large B cell lymphoma (DLBCL), mantle cell lymphoma (MCL), Burkitt's lymphoma (BL), mycosis fungoides, Sezary syndrome, cutaneous T-cell lymphoma, mast cell neoplasm, medulloblastoma, nephroblastoma, solitary plasmacytoma, myelodysplastic syndrome, chronic and non-chronic myeloproliferative disorder, central nervous system tumor, pituitary adenoma, vestibular schwannoma, primitive neuroectodermal tumor, ependymoma, choroid plexus papilloma, polycythemia vera, thrombocythemia, idiopathic myelofibrosis, and pediatric cancers such as pediatric sarcomas (e.g., neuroblastoma, rhabdomyosarcoma, and osteosarcoma). In addition, tumors can be malignant (e.g., cancers) or benign (e.g., hyperplasia, cyst, pseudocyst, hamartoma, and benign neoplasm).

Other conditions that may be treated by the antibodies and antigen-binding fragments described herein include inflammatory conditions such as rheumatoid arthritis, psoriasis, scleroderma, chronic obstructive pulmonary disease, and asthma.

In other non-therapeutic embodiments, the antibodies or antigen-binding fragments may be used in various in vitro or in vivo diagnostic or detection applications.

The antibodies disclosed herein may be administered alone or in combination with one or more additional therapeutic agents. For example, the antibodies or antigen-binding fragments disclosed herein may be administered in combination with chemotherapy, radiation therapy and cancer surgery. Furthermore, the antibodies or antigen-binding

fragments disclosed herein may be administered in combination with treatments for complications arising from chemotherapy or other therapeutic agents. In certain embodiments, the antibodies or antigen-binding fragments disclosed herein may be administered as part of the same pharmaceutical composition.

5 In certain embodiments, conjugates linked to the antibodies or antigen-binding fragments disclosed herein may comprise one or more agents meant to alter one or more pharmacokinetic (PK) properties of the antibody or antigen-binding fragment, such as for example polyethylene glycol (PEG) to increase the half-life.

In certain embodiments, compositions are provided comprising antibodies disclosed
10 herein in combination with one or more cytokines. Example of cytokines include but are not limited to lymphokines, monokines, human growth hormone, N-methionyl human growth hormone, bovine growth hormone, parathyroid hormone, thyroxine, insulin, proinsulin, relaxin, prorelaxin, follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), luteinizing hormone (LH), hepatic growth factor, fibroblast growth factor,
15 prolactin, placental lactogen, tumor necrosis factor .alpha. and .beta., mullerian-inhibiting substance, mouse gonadotropin-associated peptide, inhibin, activin, integrin, thrombopoietin (TPO), nerve growth factors such as NGF-.beta., platelet growth factor, transforming growth factors 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 such as macrophage-CSF, granulocyte macrophage CSF, and granulocyte-CSF, interleukins such IL-1, IL-1.alpha., IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, and IL-12, tumor necrosis factors such as TNF-.alpha. and TNF-.beta., and other polypeptide factors. The antibodies or antigen-binding fragments disclosed herein may be provided and/or administered in
20 combination with any cytokine, including any of those listed above.

In certain embodiments, compositions are provided comprising antibodies or antigen-binding fragments disclosed herein linked to or in combination with one or more chemotherapeutic agents. Examples of chemotherapeutic agents include, but are not limited to, ALT-110, AMN-107 (Nilotinib), amrubicin, ARQ-197, atrasentan
30 (Xinlay.RTM.), AV-299, AZD 1152, AZD 2171, batabulin, BIO-111, BIO-140, calcitriol, CC 8490, cilengitide, dasatinib, decatanib, DN-101, edotecarin, enzastaurin, erlotinib, everolimus, gimatecan, gossypol (e.g., gossypol acetate), GSK461364, GSK690693, IL13-PE38QQR, INO 1001, IPdR, ipilimumab, KRX-0402, Lep-etu, lonafarnib, lucanthone, LY

317615, MK-0457, MLN8054, neuradiab, nolatrexed, oblimersen, ofatumumab, ON
0910.Na, oregovomab, panitumumab, pazopanib, PHA-739358, R-763, RTA 744,
rubitecan, Sdx 102, talampanel, temsirolimus, tesmilifene, tetrandrine, ticilimumab, TKI-
258, TLK 286, trabectedin, vandetanib, vitespan, Xr 311, zanolimumab, 131-I-TM-601,
5 and zolendronate, histrelin, azacitidine, dexrazoxane, alemtuzumab, lenalidomide,
gemtuzumab, ketoconazole, nitrogen mustard, ibritumomab tiuxetan, decitabine,
hexamethylmelamine, bexarotene, tositumomab, arsenic trioxide, editronate, cyclosporine,
Edwina-asparaginase, strontium 89, romidepsin (FK-228), ADS-100380, CG-781, CG-
1521, IMT504, IMT506, IMT507, SB-556629, chlamydocin, vorinostat, etoposide,
10 gemcitabine, doxorubicin, 5'-deoxy-5-fluorouridine, vincristine, temozolomide,
camptothecin, irinotecan, oxaliplatin, tamoxifen, anastrozole, diethylstilbestrol,
Bevacizumab, leuprolide, sunitinib, medroxyprogesterone, raloxifene, bicalutamide,
flutamide, amifostine, NVP-LAQ824, suberoyl analide hydroxamic acid, valproic acid,
trichostatin A, FK-228, SU11248, BAY43-9006, KRN951, aminoglutethimide, amsacrine,
15 anagrelide, anastrozole, asparaginase, bacillus Calmette-Guerin (BCG) vaccine,
bleomycin, buserelin, busulfan (1,4-butanediol dimethanesulfonate), satraplatin,
carboplatin, carmustine, chlorambucil, cisplatin, cladribine, clodronate, cyclophosphamide,
cyproterone, cytarabine, dacarbazine, dactinomycin, daunorubicin, diethylstilbestrol,
epirubicin, fludarabine, fludrocortisone, fluoxymesterone, flutamide, hydroxyurea,
20 idarubicin, ifosfamide, imatinib, leucovorin, leuprolide, levamisole, lomustine,
mechlorethamine, melphalan, mercaptopurine, mesna, methotrexate, mitomycin, mitotane,
mitoxantrone, nilutamide, octreotide, edotreotide, oxaliplatin, pamidronate, Pentostatin,
plicamycin, porfimer, procarbazine, raltitrexed, rituximab, streptozocin, teniposide,
testosterone, thalidomide, thalidomide combined with dexamethasone, thioguanine,
25 thiotepa, tretinoin, vindesine, all trans-retinoic acid, or 13-cis-retinoic acid, phenylalanine
mustard, uracil mustard, estramustine, altretamine, floxuridine, 5-deoxyuridine, cytosine
arabinoside, 6-mercaptopurine, deoxycoformycin, calcitriol, valrubicin, mithramycin,
vinblastine, vinorelbine, topotecan, razoxin, marimastat, COL-3, neovastat, BMS-275291,
squalamine, endostatin, SU5416, SU6668, EMD121974, interleukin-12, IM862,
30 angiostatin, vitaxin, droloxifene, idoxifene, spironolactone, finasteride, cimitidine,
trastuzumab, denileukin, diftitox, gefitinib, bortezomib, paclitaxel, docetaxel, epithilone B,
BMS-247550, BMS-310705, droloxifene (3-hydroxytamoxifen), 4-hydroxytamoxifen,
pipendoxifene, ERA-923, arzoxifene, fulvestrant, acolbifene, lasofoxifene, idoxifene, TSE-

424, HMR-3339, ZK186619, topotecan, PTK787/ZK 222584, VX-745, PD 184352, LY294002, LY292223, LY292696, LY293684, LY293646, wortmannin, BAY-43-9006, ZM336372, L-779,450, flavopiridol, UCN-01, rapamycin, everolimus.

In certain embodiments, an antibody provided herein is in combination with one or more of pegylated or unpegylated interferon alfa-2a, pegylated or unpegylated interferon alfa-2b, pegylated or unpegylated interferon alfa-2c, pegylated or unpegylated interferon alfa n-1, pegylated or unpegylated interferon alfa n-3, and pegylated, unpegylated consensus interferon or albumin-interferon-alpha.

Compositions comprising an antiemetic are useful for preventing or treating nausea; a common side effect of chemotherapy. Accordingly, in certain embodiments compositions are provided that comprise an antibody provided herein linked to or in combination with one or more anti-cancer agents and one or more antiemetics, including but not limited to casopitant, Netupitant, palonosetron, aprepitant, diphenhydramine, hydroxyzine, metoclopramide, lorazepam, alprazolam, haloperidol, droperidol, dronabinol, dexamethasone, prednisolone, methylprednisolone, prochlorperazine, granisetron, ondansetron, dolasetron and tropisetron.

Other side effects of cancer treatment include red and white blood cell deficiency. Accordingly, compositions are provided that comprise an antibody provided herein linked to or in combination with an agent that treats red and/or white blood cell deficiency such as G-CSF, pegylated G-CSF, GM-CSF, erythropoietin and pegylated erythropoietin.

In certain embodiments, compositions are provided that comprise an antibody provided herein in combination with one or more anti-hypertensive agents such as a diuretic, an adrenergic receptor antagonist, an adrenergic receptor agonist, a calcium channel blockers, an ACE inhibitor, an angiotensin II receptor antagonist, an aldosterone antagonist, a vasodilator, or a centrally acting adrenergic drug.

In certain embodiments, the antibodies disclosed herein may be administered as part of a pharmaceutical composition that comprises one or more physiologically tolerable components. Therefore, in certain embodiments, such compositions and methods of formulating such compositions are provided herein. Compositions comprising one or more antibodies disclosed herein and one or more physiologically tolerable components may be used in the treatment of diseases associated with increased angiogenesis.

Examples of physiologically tolerable components for use in the pharmaceutical compositions disclosed herein are: pharmaceutically acceptable liquid, gel, or solid

carriers, diluents, adjuvants, excipients, auxiliary substances and other components known in the art, or combinations thereof. Examples of suitable components are: antioxidants, fillers, binders, disintegrants, buffers, preservatives, lubricants, flavorings, thickeners, coloring agents, or emulsifiers. Examples of suitable antioxidants are: methionine, ascorbic acid, EDTA, sodium thiosulfate, platinum, catalase, citric acid, cysteine, thioglycerol, thioglycolic acid, thiosorbitol, butylated hydroxanisole, butylated hydroxytoluene, and propyl gallate. Examples of suitable carriers are: aqueous vehicles such as physiological sodium chloride, Ringer's solution, isotonic dextrose injection, sterile water injection, or dextrose and lactated Ringer's injection, nonaqueous vehicles such as fixed oils of vegetable origin, cottonseed oil, corn oil, sesame oil, or peanut oil, antimicrobial agents at bacteriostatic or fungistatic concentrations, isotonic agents such as sodium chloride or dextrose, buffers such as phosphate or citrate buffers, antioxidants such as sodium bisulfate, local anesthetics such as procaine hydrochloride, suspending and dispersing agents such as sodium carboxymethylcellulose, hydroxypropyl methylcellulose, or polyvinylpyrrolidone, emulsifying agents such as Polysorbate 80 (TWEEN-80), sequestering or chelating agents such as EDTA (ethylenediaminetetraacetic acid) or EGTA (ethylene glycol tetraacetic acid), ethyl alcohol, polyethylene glycol, propylene glycol, sodium hydroxide, hydrochloric acid, citric acid, or lactic acid. Antimicrobial agents utilized as carriers may be added to pharmaceutical compositions in multiple-dose containers that include phenols or cresols, mercurials, benzyl alcohol, chlorobutanol, methyl and propyl p-hydroxybenzoic acid esters, thimerosal, benzalkonium chloride and benzethonium chloride. Suitable excipients may include, for example, water, saline, dextrose, glycerol, or ethanol. Suitable non-toxic auxiliary substances may include, for example, wetting or emulsifying agents, pH buffering agents, stabilizers, solubility enhancers, or agents such as sodium acetate, sorbitan monolaurate, triethanolamine oleate, or cyclodextrin.

The term "therapeutically effective amount" or "effective dosage" as used herein refers to the dosage or concentration of a drug effective to treat a disease or condition. For example, with regard to the use of the antibodies disclosed herein to treat cancer, a therapeutically effective amount is the dosage or concentration of the antibody or antigen-binding fragment capable of eradicating all or part of a tumor, inhibiting or slowing tumor growth, inhibiting growth or proliferation of cells mediating a cancerous condition, inhibiting tumor cell metastasis, ameliorating any symptom or marker associated with a

tumor or cancerous condition, preventing or delaying the development of a tumor or cancerous condition, or some combination thereof.

The effective dosage of an antibody or antigen-binding fragment provided herein may be determined using methods well known in the art. For example, the effective dosage
5 may be established by determining whether a tumor being treated in a subject shrinks, ceases to grow, or grows more slowly following administration at a particular dosage.

In certain embodiments, an antibody as provided herein may be administered at a therapeutically effective dosage of about 0.01 $\mu\text{g}/\text{mouse}$ to about 100 $\mu\text{g}/\text{mouse}$. A given dosage may be administered at various intervals, such as for example once a day, two or
10 more times per day, two or more times per week, once per week, once every two weeks, once every three weeks, once a month, or once every two or more months. In certain embodiments, the administration dosage may change over the course of treatment. For example, in certain embodiments the initial administration dosage may be higher than subsequent administration dosages. In certain embodiments, the administration dosage may
15 vary over the course of treatment depending on the reaction of the subject.

Dosage regimens may be adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single dose may be administered, or several divided doses may be administered over time.

Pharmaceutical compositions comprising the antibodies disclosed herein, and in
20 certain embodiments in combination with chemotherapeutic agents, may be prepared by methods well known in the art.

The antibodies and antigen-binding fragments disclosed herein may be administered by any route known in the art, such as for example parenteral (e.g., subcutaneous, intraperitoneal, intravenous, including intravenous infusion, intramuscular,
25 or intradermal injection) or non-parenteral (e.g., oral, intranasal, intraocular, sublingual, rectal, or topical) routes. In embodiments wherein the antibodies are administered via injection, injectable pharmaceutical compositions may be prepared in any conventional form, such as for example liquid solution, suspension, emulsion, or solid forms suitable for generating liquid solution, suspension, or emulsion. Preparations for injection may include
30 sterile solutions ready for injection, sterile dry soluble products, such as lyophilized powders, ready to be combined with a solvent just prior to use, including hypodermic tablets, sterile suspensions ready for injection, sterile dry insoluble products ready to be

combined with a vehicle just prior to use, and sterile emulsions. The solutions may be either aqueous or nonaqueous.

In certain embodiments, a sterile, lyophilized powder is prepared by dissolving an antibody or antigen-binding fragment as disclosed herein in a suitable solvent. The solvent
5 may contain an excipient which improves the stability or other pharmacological components of the powder or reconstituted solution, prepared from the powder. Excipients that may be used include, but are not limited to, water, dextrose, sorbital, fructose, corn syrup, xylitol, glycerin, glucose, sucrose or other suitable agent. The solvent may contain a buffer, such as citrate, sodium or potassium phosphate or other such buffer known to those
10 of skill in the art at, in one embodiment, about neutral pH. Subsequent sterile filtration of the solution followed by lyophilization under standard conditions known to those of skill in the art provides a desirable formulation. In one embodiment, the resulting solution will be apportioned into vials for lyophilization. Each vial can contain a single dosage or multiple dosages of the anti-VEGF antibody or composition thereof.

15 Reconstitution of a lyophilized powder with water for injection provides a formulation for use in parenteral administration. In one embodiment, for reconstitution the lyophilized powder is added to sterile water or other liquid suitable carrier. The precise amount depends upon the selected therapy being given, and can be empirically determined.

- In certain embodiments, systems and methods are provided for production of the
20 antibodies disclosed herein. The mouse antibody mMCA-AB1-anti-VEGF-A of this invention can be produced by culturing the cell clone (hybridoma) producing mMCA-AB1-anti-VEGF-A, in vitro or in vivo, using standard techniques well known in the art (See for example Birch JR et al 1985). Alternatively, an expression system can be used. DNA encoding the parental mouse monoclonal antibody of this invention, can be
25 isolated from the producer cells or synthesized in vitro, inserted in a cloning vector and introduced into a suitable host cell or bacteria using standard procedures (Davies SL. 2009, Volume 6, 153-173).

Non- natural versions of the mouse parental antibody disclosed herein (e.g. chimeric antibodies, humanized antibodies, or single chain antibodies) can be prepared
30 using methods well known in the art (Morrison SL et al 1984; Neuberger MS et al 1984; Takeda S et al 1985). Expression systems can be also used to produce the different antibody derivatives of the mouse parental monoclonal antibody disclosed herein. These expression systems include polynucleotides encoding the antibodies, vectors comprising

these polynucleotides, and host cells comprising these vectors. Polynucleotides encoding the antibodies disclosed herein may be isolated or synthesized using methods well known in the art, and inserted into a replicable vector for amplification or cloning.

Polynucleotides encoding variable light (V_L) and variable heavy (V_H) chains of the antibodies may be expressed from a single vector, or they may be expressed using two separate vectors, followed by in vitro assembly. In certain embodiments, they may be co-expressed from two separate vectors within the same cell and assembled intracellularly. Suitable vectors may contain various configurations of one or more regulatory sequences, such as promoters, enhancers, or transcription initiation sequences, as well as genes encoding markers for phenotypic selection. Vectors having suitable backbones for expression of the antibodies disclosed herein are known in the art (See for example Davies SL et al, 2009). In certain embodiments, the vector may contain a polynucleotide sequence encoding the constant regions of the heavy chain (C_H) and light chain (C_L) of a human IgG immunoglobulin. Alternatively, the vector may express only the V_H and V_L chains of the antibody, with the expressed polypeptide comprising an Fv fragment rather than a whole antibody. Vectors may be inserted into a suitable host cell for amplification or expression of the polynucleotide sequence. The host cells may be cultured for antibody production in a variety of media known in the art, such as for example Minimal Essential Medium (MEM) (Sigma), RPMI-1640 (Sigma), Dulbecco's Modified Eagle's Medium (DMEM) (Sigma), and Ham's F10 (Sigma). Media may be supplemented with a variety of agents, such as for example hormones, growth factors, salts, buffers, nucleotides, antibiotics, trace elements, glucose, or other energy sources. Culture conditions such as temperature and pH may be adjusted using parameters well known in the art. Following expression, one or more antibodies as provided herein may be purified using methods well known in the art (See for example Vijayalakshmi MA. 1998).

The antibodies disclosed herein may comprise conjugates for specific delivery to the tumor location.

The following examples are provided to better illustrate the claimed invention and are not to be interpreted as limiting the scope of the invention. All specific compositions, materials, and methods described below, in whole or in part, fall within the scope of the present invention. These specific compositions, materials, and methods are not intended to limit the invention, but merely to illustrate specific embodiments falling within the scope of the invention. One skilled in the art may develop equivalent compositions, materials,

and methods without the exercise of inventive capacity and without departing from the scope of the invention. It will be understood that many variations can be made in the procedures herein described while still remaining within the bounds of the present invention. It is the intention of the inventors that such variations are included within the scope of the invention.

EXAMPLES

EXAMPLE 1: Generation of Anti-VEGF Monoclonal Antibody:

Monoclonal antibodies were generated in general according to Kohler's method (Kohler G et al 1975). However, the immunization scheme was unusual. In brief 4 week-old BALB/c female mice were injected once a month for a period of 10 months with human recombinant VEGF₁₆₅ (SEQ ID NO:1) conjugated with cationized BSA (Imject® BSA and EDC Conjugation Kits, Pierce, France) in aluminum hydroxide solution (Imject® Alu, Pierce). Lymphocytes were harvested and fused with myeloma cells by treatment with polyethylene glycol (PEG 1500, BDH). After this, hybridoma cells were seeded and grown in HAT medium (hypoxanthine aminopterin thymidine, Sigma). The presence of specific antibodies was detected by direct ELISA against VEGF-A without BSA. A limited dilution method was used to select anti-humanVEGF antibody producing clones. Afterward, in order to select cell clones producing anti-humanVEGF-A antibodies of very high affinity, the HUVEC proliferation assay was used.

Using this methodology, several candidate hibridomas were isolated and frozen. One of them, named mMcA-AB1-anti-VEGF-A clone was further studied. Monoclonal antibody produced by this mMcA-AB1-anti-VEGF-A hibridoma was named mMcA-AB1-anti-VEGF-A (in short mouse AB1 antibody). Purification of this antibody was performed by standard techniques (Vijayalakshmi MA. 1998) including affinity chromatography using a protein-G Sepharose column (HiTrap Protein GHP. GE Healthcare). The mouse AB1 antibody of this invention and its derivatives do not cross-react or neutralize mouse VEGF-A as measured by ELISA and Surface Plasmon Resonance.

EXAMPLE 2: Determination of the dissociation rate constant (K_D) of the complex mMc-AB1-anti-VEGF-A/ VEGF-A₁₆₅

The affinity between the purified mouse monoclonal antibody mMc-AB1-anti-VEGF-A of this invention and VEGF-A₁₆₅ (SEQ ID NO: 1) protein was determined by

surface plasmon resonance (SPR). Affinity and Dissociation Constants of the complex mMc-AB1-anti-VEGF-A/ VEGF-A₁₆₅, K_A and K_D, respectively, were determined by kinetic analysis fitting a 1:1 interaction model using the Biacore T100 evaluation software. Results are shown in Table 1.

5

Table 1. Kinetic parameters of the interaction mMcA-AB1-anti-VEGF-A/VEGF-A₁₆₅

k _a (1/Ms)	k _d (1/s)	K _D (1/M)	K _A (M)
1.87 ± 0.02 x 10 ⁷	9.9 ± 0.4 x 10 ⁻⁵	5.3 x 10⁻¹²	1.9 x 10 ¹¹

This result indicates that the mMc-AB1-anti-VEGF-A monoclonal antibody of this invention have an unusually high (in the pM range) affinity for human VEGF-A.

10

EXAMPLE 3: Mapping of VEGF-A₁₆₅ epitopes recognized by mMc-AB1-anti-VEGF-A

Mapping of VEGF-A₁₆₅ epitopes recognized by mMc-AB1-anti-VEGF-A was performed by LC Sciences (Huston, Texas) using its PeptArrayTM system. Overlapping peptides were synthesized according to the VEGF₁₆₅ sequence (SEQ ID NO: 1). Conditions were: Binding buffer: 1X PBS, pH 7.0; Washing buffer: 1X PBS with 0.05% Tween-20 and 0.05% Triton X-100, pH 7.0. Binding condition was: 1 µg/mL mMc-AB1-anti-VEGF-A in binding buffer at 25 °C , 1 hour. Detection condition was: 10 ng/mL goat anti-mouse IgG Cy5 conjugate in binding buffer at 25 °C , 30 min. Scanning was at PMT 700 in a 635 nm channel. Results of this analysis indicated main contact of mMc-AB1-anti-VEGF-A with three VEGF-A₁₆₅ (SEQ ID NO: 1) domains:

1: HHEVVKFMDVYQRSYCH (aa 11 to 27 of VEGF-A₁₆₅)

2: YIFKPSCVPLMR (aa 44 to 56 of VEGF-A₁₆₅)

3: QIMRIKPHQGQHIG (aa 79 to 92 of VEGF-A₁₆₅)

Underlined are amino acids described as critical for binding of VEGF-A to its receptor VEGFR-2 (Kiba A et al 2003).

EXAMPLE 4: Cross-reactivity of mMcA-AB1-anti-VEGF-A with human VEGF-B₁₈₆ (SEQ ID NO: 2)

mMcA-AB1-anti-VEGF-A (6.30-0.18 µM) was injected over immobilized Vascular endothelial growth factor antigen B (VEGF-B₁₈₆) (4000 RU). K_A and K_D

30

respectively, were determined by kinetic analysis fitting a 1:1 interaction model using biacore T100 Evaluation software. Kinetic parameters and constants are shown in **Table 2**.

Table 2. Kinetic parameters of the interaction between mMCA-AB1-anti-VEGF-A and VEGF-B₁₈₆.

k_a (1/Ms)	k_d (1/s)	K_D (1/M)	K_A (M)
$32.80 \pm 0.06 \times 10^4$	$5.71 \pm 0.02 \times 10^{-3}$	1.8×10^{-7}	5.6×10^6

This result indicates that mMCA-AB1-anti-VEGF-A is able to cross-react with VEGF-B₁₈₆ albeit with low affinity as compared with its affinity for VEGF-A.

10 **EXAMPLE 5: Neutralization activity of mMCA-AB1-anti-VEGF-A on the VEGF-A induced proliferation of HUVEC in culture.**

HUVEC cells were seeded in 96 well plates at a density of 2500 cells/well containing 50 ul of M199 media supplemented with 10% FCS and 50 ug/ml gentamicin. After 2 h, 100 ul of a solution containing 25 ul of purified mMCA-AB1-anti-VEGF-A in
 15 RPMI media and 75 ul of M199 complete media containing VEGF (20 ng/ml) were added to each well. After 24 h incubation 25 ul of M199 complete media containing 2,5 μ Ci/ml methyl-[³H]-thymidine was added and incubation continued for additional 48 h. After this, 50 ul of 6 M guanidinium chloride was added to stop the reaction. Cellular brakeage was completed with three frozen-thaw cycles, DNA was collected in Whatman-GFC filters and
 20 radioactivity measured. Fig 1 shows a plot comparing the neutralization capacity of the mMCA-AB1-anti-VEGF-A monoclonal antibody of this invention and the neutralization capacity of the anti-VEGF monoclonal antibody Bevacizumab in the art (Zondor SD et al 2004).

25 **EXAMPLE 6: Variable Heavy and Variable Light chains sequences of mMCA-AB1-anti-VEGF-A**

To obtain the sequence of the genes codifying the variable heavy and light chains of mMCA-AB1-anti-VEGF-A, total RNA was extracted from hybridoma cells producing the antibody by the TRIzol (Gibco) method. The cDNA was synthesized using reverse

transcriptase (Promega) with random primers (Invitrogen). Amplification of the variable heavy and light chains of the Fab region was carried out as described (Sastry L. 1989).

Primers used for amplification of the variable heavy chain (5' end):

- CPV 1. 5'-AGGT(C/G)(C/A)A(G/A)CT(G/T)CTCGAGTC(T/A)GG-3'
- 5 CPV 2. 5'-AGGTCCAGCTGCTCGAGTCTGG-3'
- CPV 3. 5'-AGGTCCAGCTGCTCGAGTCAGG-3'
- CPV 4. 5'-AGGTCCAGCTTCTCGAGTCTGG-3'
- CPV 5. 5'-AGGTCCAGCTTCTCGAGTCAGG-3'
- CPV 6. 5'-AGGTCCAACCTGCTCGAGTCTGG-3'
- 10 CPV 7. 5'-AGGTCCAACCTGCTCGAGTCAGG-3'
- CPV 8. 5'-AGGTCCAACCTTCTCGAGTCTGG-3'
- CPV 9. 5'-AGGTCCAACCTTCTCGAGTCAGG-3'
- CPV 10. 5'-AGGTCCGAACCTTCTCGAGTC(T/A)GG-3'

Primer used for amplification of the variable heavy chain (3' end):

- 15 :5'-AGGCTTTACTAGTACAATCCCTGGGCACAATT-3'

Primers used for amplification of the variable light chain (5' end):

- CLV 1. 5'-CCAGTTCCGAGCTCGTGCTCACCCAGTCTCCA-3'
- CLV 2. 5'-CCAGTTCCGAGCTCCAGATGACCCAGTCTCCA-3'
- CLV 3. 5'-CCAGATGTGAGCTCGTGATGACCCAGACTCCA-3'
- 20 CLV 4. 5'-CCAGATGTGAGCTCGTCATGACCCAGTCTCCA-3'
- CLV 5. 5'-CCAGTTCCGAGCTCGTGATGACACAGTCTCCA-3'

Primer used for amplification of the variable heavy chain (3' end):

5'-GCGCCGTCTAGAATTAACACTCATTCCTGTTGAA-3'

- 25 The resulting products were cloned into the pComb 3 vector (Barbas, C.F. 1991) and sequenced. The deduced amino acid sequences of the variable heavy and light chains of the mouse anti-VEGF AB1 monoclonal antibody of the invention results as follows:

Variable Heavy

- QVKLLES~~GP~~ELKKPGETVKISCKASGYTFTNFGMNWVKQAPGKGLKWMGWINTN
TGEPTYVDDFKGRFAFSLETSASSAYLQISNLNNEDTATYFCARYYGSTSVWYFDV
 30 WGAGTTVTVSS (SEQ ID NO: 3)

Variable Light

ELVMTQTPSSLSASLGDRVTITCRASQDIFNYLNWYQQKPDGPIKLLIYYSSRLHSG
VPSRFSGSGSGTDYSLTISNLDREDIATYFCQQGFTLPWTFGGGGTKLEIKR (SEQ ID
NO: 4)

Note: CDR regions are underlined

5

EXAMPLE 7: Humanization of mMcA-AB1-anti-VEGF-A

For mMcA-AB1-anti-VEGF-A humanization, the framework regions between CDRs were replaced for the most homologous framework regions obtained from IgG genes of human germinal cell lines (Tan P et al 2002). This methodology introduces minimal mouse amino acid residues in order to avoid immunological reactivity during treatment of humans. For homology studies the used data base was IMGT (www.imgt.org). Designed genes containing codifying CDRs of mMcA-AB1-anti-VEGF-A and framework regions of IgG genes of human germinal cell lines as well as a secretion signal were synthesized by a commercial synthesis service (GenScript Corporation, www.genscript.com). The synthetic gene corresponding to the humanized variable heavy region was inserted into the EcoRI and NheI sites of the pFUSE-CHlg-hG1 vector (InvivoGen) and the synthetic gene corresponding to the humanized variable light region was inserted into the BtsEII and BsiWI sites of the pFUSE2-CLlg-hk vector (InvivoGen). Both vectors provide the human constant regions necessary to complete the heavy (pFUSE-CHlg-hG1) and the light (pFUSE2-CLlg-hk) human IgG chains in order to obtain the humanized antibody. Furthermore, plasmids pFUSE-CHlg-hG1-P and pFUSE2-CLlg-hk-P were cotransfected into NS0 cells using Lipofectamine 2000 (Invitrogen). Cell clones expressing anti-VEGF antibody activity were selected and one of them used for antibody production and purification using methods well known in the art.

25 The Variable Heavy and Variable Light chains sequences of the humanized version of mMcA-AB1-anti-VEGF-A are as follows:

Variable Heavy:

QVQLVQSGSELKKPGASVKVSCKASGYTFTNFGMNWVRQAPGQGLEWMGWINT
NTGEPTYVDDEFKGRFVFSLDTSVSTAYLQISSLKAEDTAVYYCARYYGSTSVWYF
30 DWWGRGTLVTVSS (SEQ ID NO: 5)

Variable Light:

DIQMTQSPSSLSASVGDRVTITCRASQDIFNYLNWYQQKPGKAPKLLIYYSSRLHS
 GVPSRFSGSGSGTDFTFTISSLQPEDATYYCQQGFTLPWTFGQGTKVEIKR (SEQ
 ID NO: 6)

Note: CDR regions are underlined

5

EXAMPLE 8: Determination of the dissociation rate constant (K_D) of the complex humanized version of mMc-AB1-anti-VEGF-A/ VEGF-A₁₆₅

The affinity between the humanized version of mMcA-AB1-anti-VEGF-A and VEGF-A₁₆₅ (SEQ ID NO: 1) was determined by surface plasmon resonance (SPR). Affinity and
 10 Dissociation Constants, K_A and K_D , respectively, were determined by kinetic analysis fitting a 1:1 interaction model using the BIAcore T100 evaluation software. Results are shown in **Table 3**.

Table 3. Kinetic parameters of the interaction humanized mMc-AB1-anti-VEGF-A/ VEGF-A₁₆₅

15

k_a (1/Ms)	k_d (1/s)	K_D (1/M)	K_A (M)
$3.83 \pm 0.03 \times 10^6$	$4.21 \pm 0.02 \times 10^{-4}$	1.09×10^{-10}	9.24×10^5

EXAMPLE 9: Neutralization activity of humanized mMcA-AB1-anti-VEGF-A on the VEGF-A induced proliferation of HUVEC in culture.

HUVEC cells were seeded in 96 well plates at a density of 2500 cells/well
 20 containing 50 ul of M199 media with addition of 10% FCS and 50 ug/ml gentamicin. After 2 h, 100 ul of a solution containing 25 ul of purified antibody in RPMI media and 75 ul of M199 complete media containing VEGF (20 ng/ml) were added to each well. After 24 h incubation 25 ul of M199 complete media containing 2,5 μ Ci/ml methyl-[³H]-thymidine was added and incubation continued for additional 48 h. After this, 50 ul of 6 M
 25 guanidinium chloride were added to stop the reaction. Cellular brakeage was completed with three cycles of frozen-thaw DNA was collected in Whatman GFC filters and radioactivity measured. Fig 2 shows a plot comparing the neutralization capacity of the

humanized mMcA-AB1-anti-VEGF-A on the VEGF-A induced proliferation of HUVEC in culture. and the neutralization capacity of the anti-VEGF monoclonal antibody Bevacizumab in the art.

5 **EXAMPLE 10: In vivo Activity of the Anti-VEGF Antibodies**

IIB-Mel-J cells (Guerra, L., et al., Characterization of IIB-MEL-J: a new and highly heterogeneous human melanoma cell line. *Pigment Cell Res*, 1989. 2(6): p. 504-9) were used to study the tumor growth inhibition in nude mice. IIB-Mel-J cells were maintained in culture with DMEM medium, supplemented with 10% FBS and 5 µg/ml insulin and 50 µg/ml gentamycin. Cells were grown at 37° C, in 5% CO₂ until confluence, harvested, counted, washed and resuspended in sterile Matrigel at a concentration of 50 x 10⁶ cell/ml. Xenografts were established in 4-6 week-old Nude Swiss mice by injecting 5x 10⁶ IIB-Mel-J cells into the dorsal flank of the mice. After 6 days were tumors were palpable, mice were randomly distributed into 10 groups (n=10) and intraperitoneally injected twice weekly with either PBS (control group) or different antibodies in PBS in three different concentrations each as follows: group A, mice injected with 0.1 ml of PBS; group B, mice injected with 0.1 ml of the AB1 antibody at a dose of 1 µg/mouse; group C, mice injected with 0.1 ml of the AB1 antibody at a dose of 25 µg/mouse; group D, mice injected with 0.1 ml of the humanized version of AB1 antibody at a dose of 1 µg/mouse; group E, mice injected with 0.1 ml of the humanized version of AB1 antibody at a dose of 25 µg/mouse; group F, mice injected with 0.1 ml of the humanized version of Bevacizumab (AvastinTM) at a dose of 1 µg/mouse and group G, mice injected with 0.1 ml of the AvastinTM antibody at a dose of 25 µg/mouse. Tumor growth was followed measuring length and width with a caliber instrument and the volume estimated with the following formula: $\frac{1}{2}$ length x width².

Animals were killed at day 40 and tumors excised and weighed. The results show that there was a significant inhibition of tumor growth when both, AB1 and the humanized version of AB1 antibodies were administered in all the assayed doses to mice in comparison with mice injected with PBS (p< 0.001) (Fig 3). On the other hand, tumors of animals treated with the AB1 and humanized AB1 antibodies were, after 40 days of treatment, in average smaller than tumors treated with Bevacizumab at both of the assayed doses (Fig 4). Comparison between the humanized version of AB1 and Bevacizumab are shown in Fig.5. At the 25 µg/mouse differences between these two antibodies were

statistically significant ($p < 0.001$). It is worth mention that all the assayed antibodies lack significant neutralizing activity to mouse VEGF (Liang W, Wu X, Peale FV et al. Cross-species Vascular Endothelial Growth factor (VEGF)-blocking Antibodies Completely Inhibit the Growth of Human Tumor Xenografts and Measure the Contribution of Stromal VEGF. JBC 281: 951-961 (2006) and EXAMPLE 1) a fact that renders comparison between its anti-tumor activities meaningful.

SEQUENCES

NUMBER OF SEQ ID NOS: 12

10 SEQ ID NO: 1

LENGTH: 165

TYPE: PRT

ORGANISM: Homo sapiens

PUBLICATION INFORMATION:

15 DATA BASE ACCESSION NUMBER: GeneBank AAA35789

DATABASE ENTRY DATE: 1993-04-27

SEQUENCE:1

APMAEGGGQNHHEVVKFMDVYQRSYCHPIETLVDIFQEYPDEIEYIFKP
 SCVPLMRCGGCCNDEGLECVPTESNITMQIMRIKPHQGQHIGEMSFLQ
 20 HNKCECRPKKDRARQENPCGPCSERRKHLFVQDPQTCKCCKNTDSRCK
 ARQLELNERTCRCDKPRR

SEQ ID NO: 2

LENGTH: 186

25 TYPE: PRT

ORGANISM: Homo sapiens

PUBLICATION INFORMATION:

DATA BASE ACCESSION NUMBER: GeneBank AAC50721.1

DATABASE ENTRY DATE: 1996-08-12

30 SEQUENCE: 2

PVSQPDAPG HQRKVVSVIDVYTRATCQPR EVVVPLTVELMGTVAKQLVP
 SCVTVQRCGG CCPDDGLECV PTGQHQVRMQ ILMIRYPSSQLGEMSLEEHS

27

QCECRPKKKD SAVKPDRAAT PHHRPQPRSV PGWDSAPGAPPADITHPTP
APGPSAHAAPSTTSALTPGP AAAAADAAAS SVAKGGA

SEQ ID NO: 3

5

LENGTH: 121

TYPE: PRT

ORGANISM: Mus musculus

OTHER INFORMATION: antibody heavy chain variable region

SEQUENCE: 3

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WYFDVWGAGTTVTVSS

SEQ ID NO: 4

15

LENGTH: 108

TYPE: PRT

ORGANISM: Mus musculus

OTHER INFORMATION: anti-VEGF light variable region

SEQUENCE: 4

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HSGVPSRFSGSGSGTDYSLTISNLDREDIATYFCQQGFTLPWTFGGGTKLEIKR

SEQ ID NO: 5

LENGTH: 121

25

TYPE: PRT

ORGANISM: Artificial sequence

OTHER INFORMATION: recombinant anti-VEGF heavy chain variable region

SEQUENCE: 5

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VWYFDVWGRGTLTVSS

SEQ ID NO: 6

28

LENGTH: 108

TYPE: PRT

ORGANISM: Artificial sequence

OTHER INFORMATION: recombinant anti-VEGF antibody light variable region

5 SEQUENCE: 6

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HSGVPSRFSGSGSGTDFTFTISLQPEDATYYCQQGFTLPWTFGQGTKVEIKR

SEQ ID NO: 7

LENGTH: 10

10 TYPE: PRT

ORGANISM: Mus musculus

OTHER INFORMATION: CDR region. Subsequence of SEQ ID NO: 3

SEQUENCE: 7

GYTFTNFGMN

15 SEQ ID NO: 8

LENGTH: 17

TYPE: PRT

ORGANISM: Mus musculus

OTHER INFORMATION: CDR region. Subsequence of SEQ ID NO: 3

20 SEQUENCE: 8

WINTNTGEPTYVDDFKG

SEQ ID NO: 9

LENGTH: 12

25 TYPE: PRT

ORGANISM: Mus musculus

OTHER INFORMATION: CDR region. Subsequence of SEQ ID NO: 3

SEQUENCE: 9

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30

SEQ ID NO: 10

LENGTH: 11

TYPE: PRT

29

ORGANISM: Mus musculus

OTHER INFORMATION: CDR region. Subsequence of SEQ ID NO: 4

SEQUENCE: 10

RASQDIFNYLN

5

SEQ ID NO: 11

LENGTH: 6

TYPE: PRT

ORGANISM: Mus musculus

10 OTHER INFORMATION: CDR region. Subsequence of SEQ ID NO: 4

SEQUENCE: 11

YSSRLH

SEQ ID NO: 12

15 LENGTH: 9

TYPE: PRT

ORGANISM: Mus musculus

OTHER INFORMATION: CDR region. Subsequence of SEQ ID NO: 4

SEQUENCE: 12

20 QQGFTLPWT

CLAIMS

1. An isolated antibody or antigen-binding fragment thereof that specifically binds human VEGF-A and VEGF-B and comprises a light chain immunoglobulin comprising CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 10,
5 CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 11 and CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 12 and a heavy chain immunoglobulin comprising CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 7, CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 8 and CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 9.
- 10 2. The antibody or antigen-binding fragment thereof as recited in claim 1, comprising a light chain immunoglobulin comprising the amino acid sequence set forth in SEQ ID NO:4; and a heavy chain immunoglobulin comprising the amino acid sequence set forth in SEQ ID NO:3.
- 15 3. The antibody or antigen-binding fragment thereof as recited in claim 1, comprising a light chain immunoglobulin comprising the amino acid sequence set forth in SEQ ID NO:6; and a heavy chain immunoglobulin comprising the amino acid sequence set forth in SEQ ID NO:5.
- 20 4. The antibody or antigen-binding fragment thereof as recited in claim 1, wherein the antibody or antigen-binding fragment thereof is selected from the group consisting of a monoclonal antibody, a polyclonal antibody, a chimeric antibody, a humanized antibody, a recombinant antibody, a fully human antibody, a bivalent antibody, an anti-idiotypic antibody, a camelized single domain antibody, a diabody, a scFv, an scFv dimer, a dsFv, a (dsFv).sub.2, a dsFv-dsFv', an Fv fragment, a Fab, a Fab', a F(ab').sub.2, a ds diabody, a nanobody, a domain
25 antibody, and a bivalent domain antibody.
5. The antibody or antigen-binding fragment thereof as recited in claim 1, further comprising an immunoglobulin constant region selected from the group consisting of a .kappa. light chain, a .gamma.1 heavy chain, a .gamma.2 heavy chain, a .gamma.3 heavy chain, and a .gamma.4 heavy chain constant region.
- 30 6. The antibody or antigen-binding fragment thereof as recited in claim 1, wherein said antibody or antigen-binding fragment inhibits tumor growth in a greater extent than Bevacizumab when administered at the same dosage.

7. The antibody or antigen-binding fragment thereof as recited in claim 1, wherein said antibody or antigen-binding fragment binds human VEGF.sub.165 with a K.sub.D of no more than 10^{-10}
8. A pharmaceutical composition comprising the antibody or antigen-binding fragment thereof as recited in claim 1 and one or more antioxidants.
9. A pharmaceutical composition comprising the antibody or antigen-binding fragment thereof as recited in claim 1 and one or more pharmaceutically acceptable carriers.
10. The pharmaceutical composition of claim 10, wherein said one or more antioxidants are selected from the group consisting of methionine, ascorbic acid, EDTA, sodium thiosulfate, platinum, catalase, citric acid, cysteine, thioglycerol, thioglycolic acid, thiosorbitol, butylated hydroxyanisole, butylated hydroxytoluene, and/or propyl gallate.
11. A pharmaceutical composition comprising the antibody or antigen-binding fragment thereof as recited in claim 1 in combination with one or more additional chemotherapeutic agents selected from the group consisting of capecitabine, a combination of irinotecan, 5-fluorouracil and leucovorin, carboplatin, leucovorin, oxaliplatin and 5-fluorouracil.
12. A method of inhibiting angiogenesis or treating a disease in a human subject in need thereof comprising administering to said subject a therapeutically effective amount of the antibody or antigen-binding fragment thereof as recited in claim 1, optionally linked to or in combination with one or more additional chemotherapeutic agents.
13. A method according to claim 12, wherein the disease is associated with aberrant angiogenesis.
14. The method according to claim 12, wherein the disease is an inflammatory disease associated with VEGF signaling or is rheumatoid arthritis.
15. A method of claim 12 wherein the disease is a wet acute macular degeneration or diabetic retinopathy.
16. A method according to claim 12 wherein the disease is a cancer associated with increased VEGF signaling.
17. A method according to claim 16 wherein the disease said cancer is breast cancer.

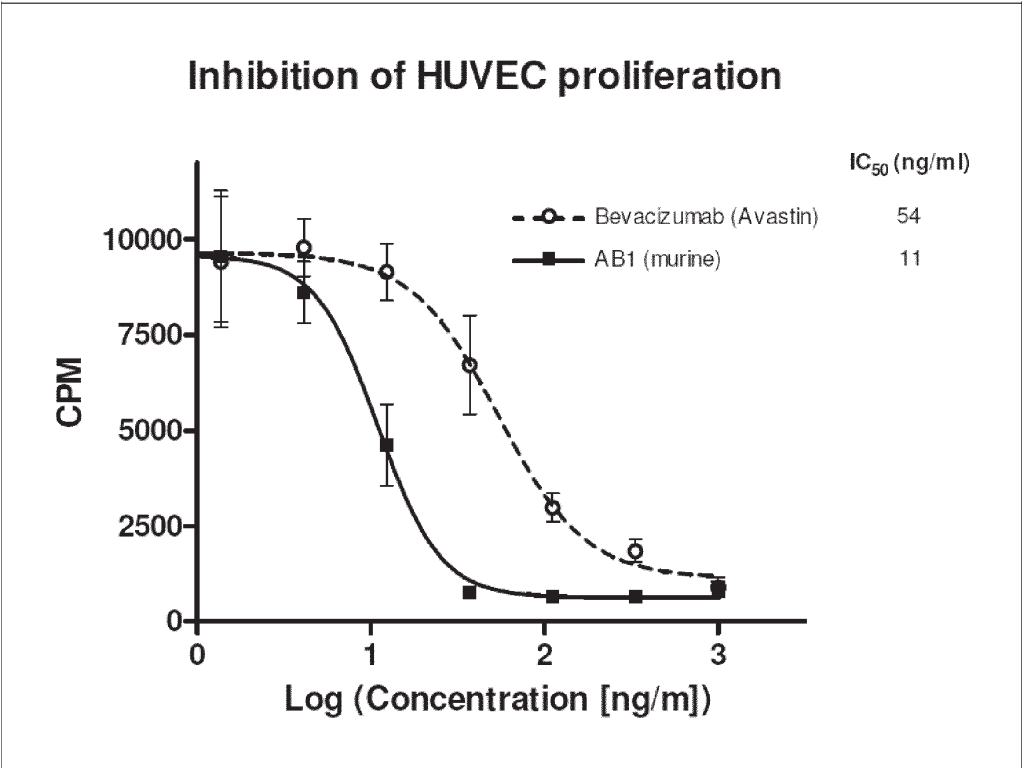


FIG. 1

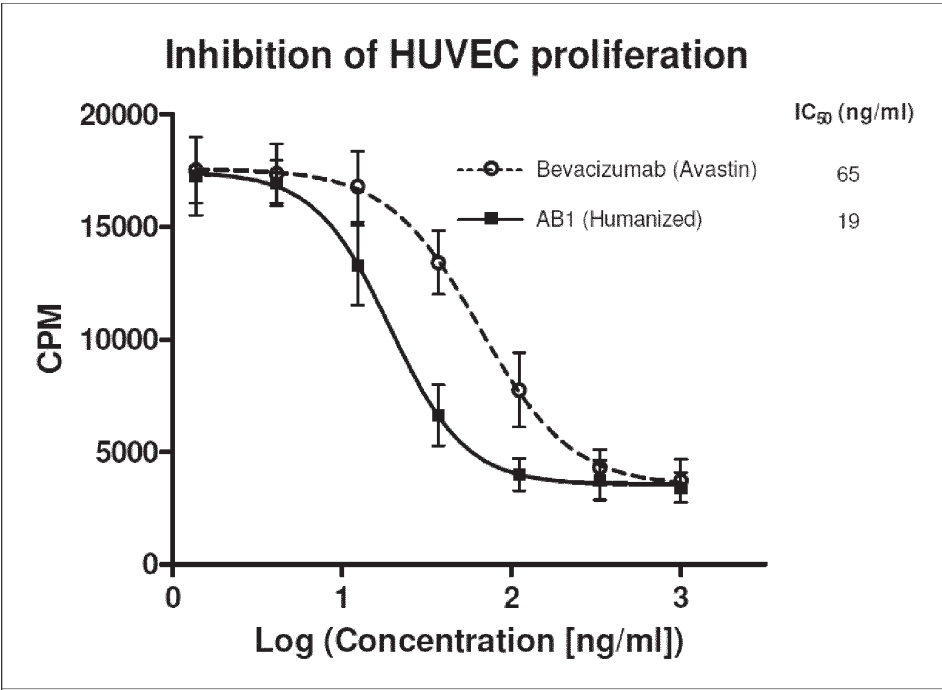


FIG. 2

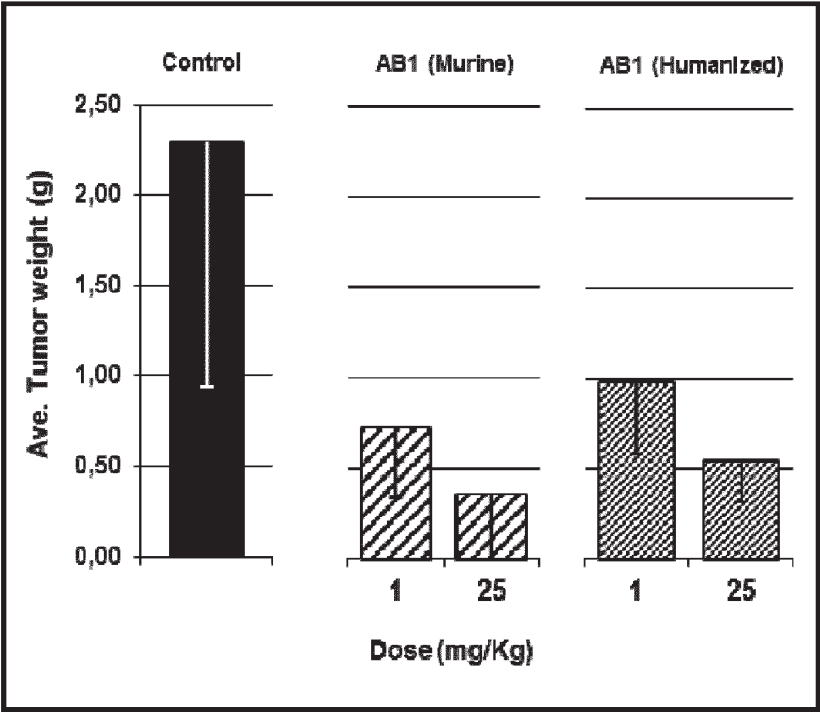


FIG. 3

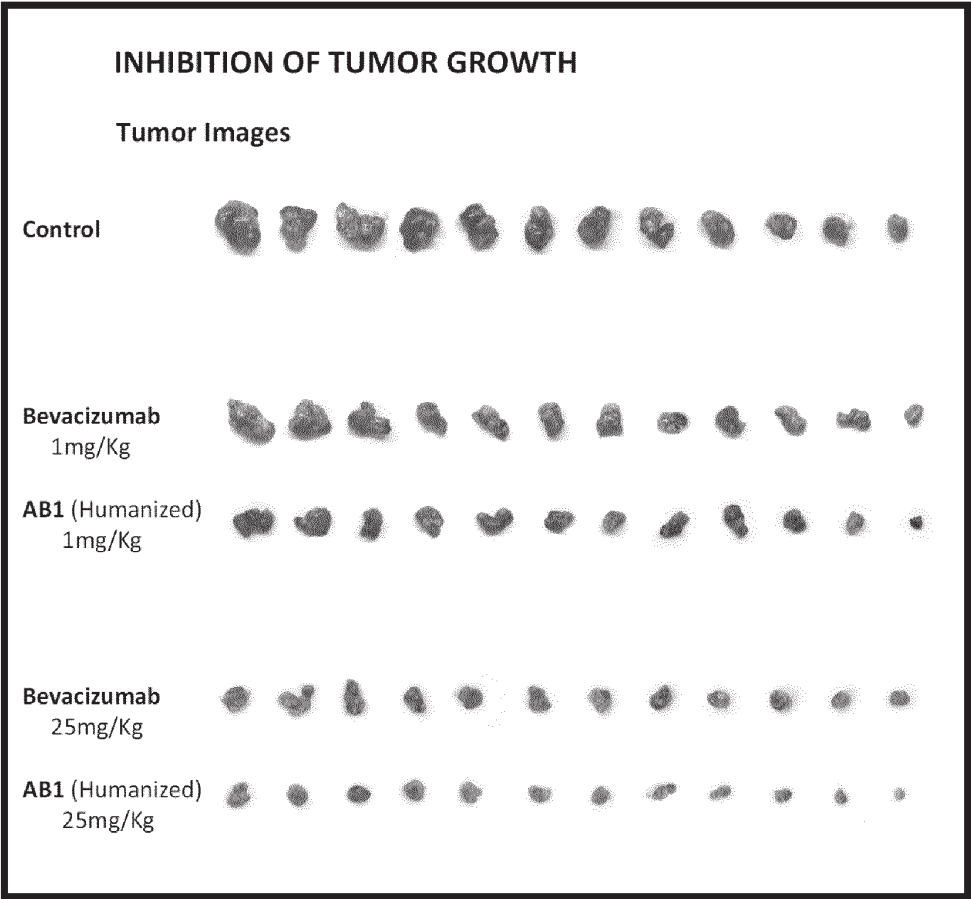


FIG. 4

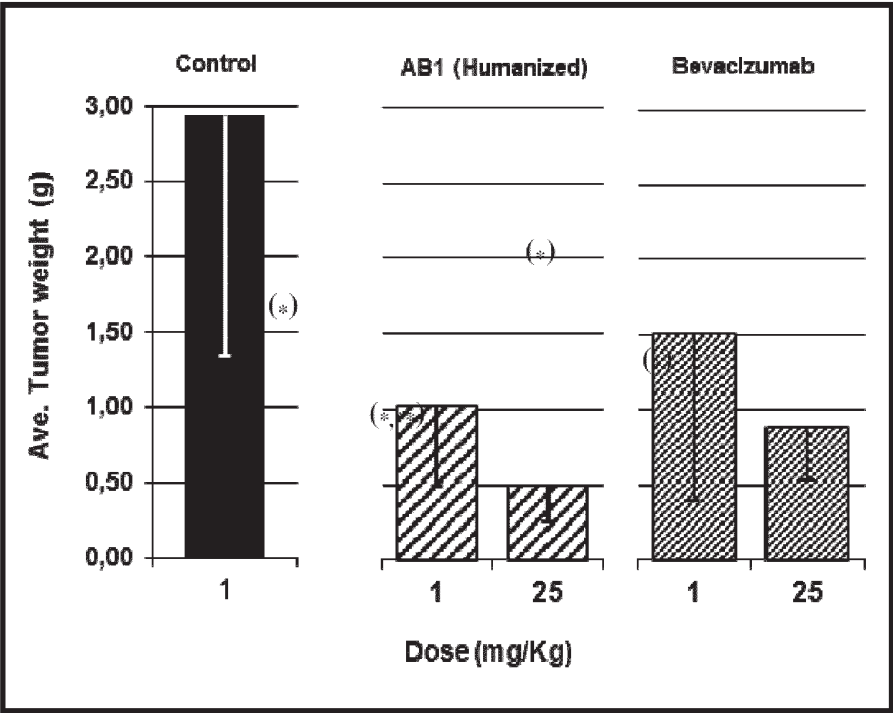


FIG. 5

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP2015/059757

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

a. ☒ forming part of the international application as filed:

☒ in the form of an Annex C/ST.25 text file.

☐ on paper or in the form of an image file.

b. ☐ furnished together with the international application under PCT Rule 13~~ter~~.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

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2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

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A. CLASSIFICATION OF SUBJECT MATTER

INV. C07K16/22

ADD. A61K39/00

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)

C07K 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 practicable, search terms used)

EPO-Internal, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2009/060198 A1 (PEREGRINE PHARMACEUTICALS INC [US]; AFFITECH AS [NO]; OWEN DEBORAH JAN) 14 May 2009 (2009-05-14) 24, 23, 20, 19, 17, 11, 12; figures 24, 23, 20, 19, 17, 11, 12; examples 5, 11-15 -& LAURA A. SULLIVAN ET AL: "r84, a Novel Therapeutic Antibody against Mouse and Human VEGF with Potent Anti-Tumor Activity and Limited Toxicity Induction", PLOS ONE, vol. 5, no. 8, 6 August 2010 (2010-08-06), page e12031, XP055201834, DOI: 10.1371/journal.pone.0012031 abstract -/-	1-17



Further documents are listed in the continuation of Box C.



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

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"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

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"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

20 July 2015

Date of mailing of the international search report

05/08/2015

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Authorized officer

Lechner, Oskar

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2015/059757

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	WO 2010/124009 A2 (SCHERING CORP [US]; XOMA TECHNOLOGY LTD; RAMACHANDRA SUMANT [US]; HUAN) 28 October 2010 (2010-10-28) claims 1-3; examples 14, 15; tables 9, 11 -----	1-17
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X	WO 2008/015239 A2 (PIERIS AG [DE]; JENSEN KRISTIAN [DE]; HUELSMEYER MARTIN [DE]; SCHLEHUB) 7 February 2008 (2008-02-07) figures 30, 35; examples 40, 45 -----	1-17
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INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2015/059757

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	HUMBERTO LAMDAN ET AL: "Affinity maturation and fine functional mapping of an antibody fragment against a novel neutralizing epitope on human vascular endothelial growth factor", MOLECULAR BIOSYSTEMS, vol. 9, no. 8, 1 January 2013 (2013-01-01), page 2097, XP055200984, ISSN: 1742-206X, DOI: 10.1039/c3mb70136k abstract -----	1-17
A	LIANG WEI-CHING ET AL: "Cross-species vascular endothelial growth factor (VEGF)-blocking antibodies completely inhibit the growth of human tumor xenografts and measure the contribution of stromal VEGF", JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY FOR BIOCHEMISTRY AND MOLECULAR BIOLOGY, US, vol. 281, no. 2, 7 November 2005 (2005-11-07), pages 951-961, XP002373804, ISSN: 0021-9258, DOI: 10.1074/JBC.M508199200 the whole document -----	1-17

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2015/059757

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CN 104278038	A	14-01-2015	NONE
