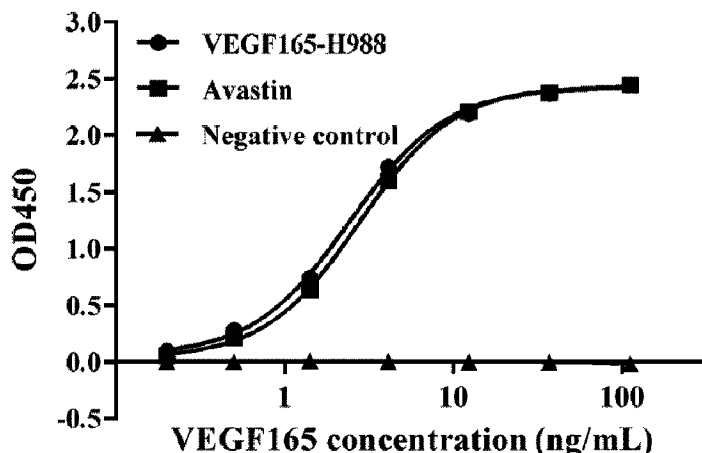




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The ELISA assay result of the binding of humanized antibody
VEGF165-H988 to VEGF165

(57) Abrégé/Abstract:

The present invention belongs to the field of tumor immunotherapy, and relates to a humanized monoclonal antibody that binds to VEGF. Provided are a nucleic acid sequence (comprising heavy/light chain variable regions) encoding the antibody, a vector comprising the nucleic acid sequence, a pharmaceutical composition and a kit. The antibody provided may specifically bind to VEGF with high affinity and block the binding of VEGF to a receptor VEGFR2. The antibody may also neutralize proliferation effects of VEGF165 alone and multiple VEGF subtypes together on HUVEC cells, and may be used for clinical treatment of tumors, comprising but not limited to: colorectal cancer.

ABSTRACT

The present invention belongs to the field of tumor immunotherapy, and relates to a humanized monoclonal antibody that
5 binds to VEGF. The present invention discloses nucleic acid sequences (including heavy/light chain variable regions) encoding said antibodies, and vectors, pharmaceutical compositions and kits containing said nucleic acid sequences. The antibody disclosed in the present invention can specifically bind to VEGF with high affinity and
10 block the binding of VEGF to the receptor VEGFR2. Said antibodies also neutralize the proliferative effect of VEGF165 protein and multiple VEGF subtypes on HUVEC cells and can be used in clinical treatment of tumors, including but not limited to: colorectal cancer.

Humanized anti-VEGF monoclonal antibody

TECHNICAL FIELD

5 The present invention relates to the field of tumor immunotherapy, specifically relates to a humanized monoclonal antibody that binds to VEGF.

BACKGROUND

10 The development of the vascular system is the foundation of many physiological and pathological processes. Vascular endothelial growth factor (VEGF) is a group of growth factors possessing important pro-angiogenic activities that promote endothelial cell mitosis and anti-apoptosis, increase vascular permeability, and promote cell migration.

15 The human VEGF gene is localized on chromosome 6p21.3 and belongs to the VEGF/PDGF supergene family, which encodes VEGF linked by disulfide bonds to form a dimer. In humans, the VEGF family includes multiple members with different functions: VEGFA (VEGF, with several different splicing variants), VEGFB, VEGFC, VEGFD,

20 VEGFE, VEGFF, and placenta growth factor (PIGF). Recently, endocrine gland-derived vascular endothelial growth factor (EG-VEGF) has been also included in this family (Samson M et al., J Clin Endocrinol Metab. 2004; 89(8):4078-4088). VEGF is widely distributed in human tissues and organs, among which the eye retinal

25 pigment epithelial cells, vascular endothelial cells, nerve cells, etc. are expressed (Goel H L et al., Nat Rev Cancer. 2013; 13(12): 871). There are three types of VEGF receptors: VEGFR1, VEGFR2 and VEGFR3. The binding of VEGF to the receptor extracellular domain triggers receptor dimerization and promote auto-phosphorylation of tyrosine

30 residues in the intracellular domain, thereby activating downstream signals that promote cell proliferation, migration, anti-apoptosis and increased vascular permeability. VEGFR1 and VEGFR2 are mainly expressed in vascular endothelial cells, while VEGFR3 is mainly expressed in lymphatic endothelial cells.

VEGF has been confirmed to play an important role in the regulation of normal and pathological angiogenesis (Melincovici C S et al., Rom J Morphol Embryol. 2018; 59(2): 455-467). VEGF is overexpressed in a variety of tumors that can cause malignant ascites, and the expression of VEGF in tumors is correlated with the migration ability of tumor cells. The concentration of VEGF in patients with solid tumors of poorer survival rate such as gastrointestinal, ovarian, breast and lung cancers is positively correlated with disease staging (Sebastian, K et al., Oncologist. 2009; 14(12): 1242 -1251). Hypoxic conditions in the tumor microenvironment induce the entry of tumor cell transcription factor HIF-1a into the nucleus, sequentially HIF-1a bind to the HRE element of VEGFA, thereby the transcription level of VEGFA is up-regulated and tumor cells is promoted to secrete large amounts of VEGF into the tumor microenvironment . In turn, the high concentration of VEGF acts on VEGFRs on vascular endothelial cells, inducing a large number of neovascularization, enhancing blood supply, and providing sufficient nutrients for the growth of tumor cells. More VEGF is secreted from the rapid-grown tumor cells, which further promotes the proliferation and migration of vascular endothelial cells and induces tumor metastasis. In addition, VEGF also stimulates monocytes in tumor tissues to convert to M2 suppressor macrophages, producing more negative immune factors, and up-regulating Treg cells at the same time, which synergistically reduce the killing ability of T cells.

By inhibiting the interaction of VEGF with endothelial cell surface receptors VEGFR2 and VEGFR1, VEGF monoclonal antibody drugs block downstream signaling pathways, inhibit endothelial cell proliferation and neovascularization, deprive tumor tissues of blood supply, and control the internal nutrient supply of tumors, thereby limiting tumor growth and ultimately achieving anticancer efficacy. Avastin (bevacizumab, approved in 2009) is the first antibody drug approved to inhibit tumor angiogenesis, and is mainly used for the treatment of breast cancer, cervical cancer, colorectal cancer, glioblastoma, glioma, non-small cell lung cancer, ovarian cancer, and renal cell carcinoma. Although Avastin has been used to treat a variety

of cancers, there is still a need in the field for more potent antibodies with greater VEGF inhibition and higher efficacy.

The present invention provides a novel human VEGF antibody for the treatment of colorectal cancer.

5

SUMMARY

In one aspect, the present invention provides an isolated anti-VEGF antibody or antigen-binding fragment thereof, comprising a heavy chain variable region having a heavy chain CDR1 region having the amino acid sequence as set forth in SEQ ID NO: 30, a heavy chain CDR2 region having the amino acid sequence as set forth in SEQ ID NO: 31 and a heavy chain CDR3 region having the amino acid sequence as set forth in SEQ ID NO: 32; and a light chain variable region having a light chain CDR1 region having the amino acid sequence as set forth in SEQ ID NO: 27, a light chain CDR2 region having the amino acid sequence as set forth in SEQ ID NO: 28, and a light chain CDR3 region having the amino acid sequence as set forth in SEQ ID NO: 29.

In one embodiment, said anti-VEGF antibody or antigen-binding fragment thereof has a heavy chain variable region having the amino acid sequence as set forth in SEQ ID NO: 39, or the amino acid sequences having at least 90%, 92%, 95%, 98% or 99% sequence identity to SEQ ID NO: 39; and a light chain variable region having the amino acid sequence as set forth in SEQ ID NO: 40, or the amino acid sequences having at least 90%, 92%, 95%, 98% or 99% sequence identity to SEQ ID NO: 40.

In one embodiment, said antibody further comprises a light chain constant region and a heavy chain constant region, preferably the light chain constant region is the light chain constant region having the amino acid sequence as set forth in SEQ ID NO: 42, or the amino acid sequences having at least 90%, 92%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 42; and/or the heavy chain constant region is the IgG1 heavy chain constant region having the amino acid sequence

as set forth in SEQ ID NO: 41, or the amino acid sequences having at least 90%, 92%, 95%, 98%, or 99% sequence identity to SEQ ID NO:41.

In one embodiment, said anti-VEGF antibody or antigen-binding fragment thereof is an IgG antibody, preferably an IgG1 antibody.

5 In one embodiment, said anti-VEGFR antibody or antigen-binding fragment thereof is a monoclonal antibody.

In one embodiment, the binding affinity K_D of said anti-VEGF antibody or antigen-binding fragment thereof to the recombinant human VEGF165 protein is 1-100 pM, preferably 5-50 pM, and more
10 preferably 19.5 pM.

In one embodiment, said antigen-binding fragment is Fv, Fab, Fab', Fab'-SH, F(ab')₂, Fd fragment, Fd' fragment, single chain antibody molecule or single domain antibody; wherein the single chain antibody molecule is preferably scFv, di-scFv, tri-scFv, diabody or scFab.

15 In another aspect, the present invention provides an antibody-drug conjugate, comprising the anti-VEGF antibody or antigen-binding fragment thereof as described herein and an additional therapeutic agent, preferably said anti-VEGF antibody or antigen-binding fragment thereof is connected with the additional therapeutic agent via a
20 connector.

In one aspect, the present invention provides an isolated anti-VEGF antibody or antigen-binding fragment thereof, comprising a heavy chain variable region having a heavy chain CDR1 region having the amino acid sequence as set forth in SEQ ID NO: 16 and a heavy chain
25 CDR2 region having the amino acid sequence as set forth in SEQ ID NO: 17 and a heavy chain CDR3 region having the amino acid sequence as set forth in SEQ ID NO: 18; and a light chain variable region having a light chain CDR1 region having the amino acid sequence as set forth in SEQ ID NO: 13, a light chain CDR2 region
30 having the amino acid sequence as set forth in SEQ ID NO: 14, and a light chain CDR3 region having the amino acid sequence as set forth in SEQ ID NO: 15.

In one embodiment, said anti-VEGF antibody or antigen-binding fragment thereof comprises a heavy chain constant region having the
35 amino acid sequence as set forth in SEQ ID NO: 25, or the amino acid

sequences having at least 90%, 92%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 25; and a light chain variable region having the amino acid sequence as set forth in SEQ ID NO: 26, or the amino acid sequences having at least 90%, 92%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 26.

In one embodiment, said anti-VEGF antibody or antigen-binding fragment thereof is a humanized antibody or a chimeric antibody.

In another aspect, the present invention provides a nucleic acid encoding the anti-VEGF antibody or antigen-binding fragment thereof as described herein.

In one embodiment, said nucleic acid comprises the nucleotide sequence as set forth in SEQ ID NO: 7 and/or the nucleotide sequence as set forth in SEQ ID NO: 8; or comprises the nucleotide sequence as set forth in SEQ ID NO: 23 and/or the nucleotide sequence as set forth in SEQ ID NO: 24; or comprises the nucleotide sequence as set forth in SEQ ID NO: 47 and/or the nucleotide sequence as set forth in SEQ ID NO: 48.

In another aspect, the present invention provides an expression vector, comprising the nucleic acid as described herein.

In another aspect, the present invention provides a host cell, comprising the nucleic acid as described herein or the expression vector as described herein.

In another aspect, the present invention provides a method for producing the anti-VEGF antibody or antigen-binding fragment thereof as described herein, comprising culturing the host cell as described herein under conditions suitable for antibody expression, and harvesting the expressed antibody from the culture medium.

In another aspect, the present invention provides a pharmaceutical composition, comprising the anti-VEGF antibody or antigen-binding fragment thereof as described herein, or the antibody-drug conjugate as described herein, or the nucleic acid as described herein, or the expression vector as described herein, and a pharmaceutically acceptable carrier.

In one embodiment, the present invention provides the anti-VEGF antibody or antigen-binding fragment thereof as described herein, or

the antibody-drug conjugate as described herein, or the pharmaceutical composition as described herein, for use in the treatment of colorectal cancer.

5 In another aspect, the present invention provides a method for treating colorectal cancer, comprising administering to a subject in need a therapeutically effective amount of the anti-VEGF antibody or antigen-binding fragment thereof as described herein, or the antibody-drug conjugate as described herein, or the pharmaceutical composition as described herein.

10 In another aspect, the present invention provides the use of the anti-VEGF antibody or antigen-binding fragment thereof as described herein or the antibody-drug conjugate as described herein or the pharmaceutical composition as described herein in the preparation of a medicament for the treatment of colorectal cancer.

15 In another aspect, the present invention provides a pharmaceutical combination, comprising the anti-VEGFR2 antibody or antigen-binding fragment thereof as described herein, or the antibody-drug conjugate as described herein, or the pharmaceutical composition as described herein, and one or more additional therapeutic agents.

20 In another aspect, the present invention provides a kit, comprising the anti-VEGF antibody or antigen-binding fragment as described herein, or the antibody-drug conjugate as described herein, or the pharmaceutical composition as described herein, preferably, further comprising a device for administration.

25

BRIEF DESCRIPTION OF THE DRAWINGS

The present invention is illustrated in combination with the attached drawings, in which:

30 Figure 1 shows that the rabbit antibody VEGF165 blocks the binding of VEGF165 to VEGFR2 protein.

Figure 2 shows that rabbit antibody VEGF165 neutralizes the VEGF165-induced the proliferation of HUVEC.

Figure 3 shows the binding of humanized antibody VEGF165-H988 to VEGF165, detected by ELISA.

Figure 4 shows the binding of humanized antibody VEGF165-H988 to mVEGF165, detected by ELISA.

Figure 5 shows that the antibody VEGF-H988 blocks the binding of VEGF165 to VEGFR2 protein, detected by ELISA.

5 Figure 6 shows the effect of antibody VEGF-H988 in neutralizing VEGF165 in different concentrations.

Figure 7 shows the effect of VEGF-H988 on the neutralization of VEGF165, VEGFC and VEGFD -induced the proliferation of HUVEC cells.

10 Figure 8 shows the mean blood concentration-time curve of G2 group mice injected with VEGF-H988 (50 mg/kg) intraperitoneally.

Figure 9 shows the mean blood concentration-time curve of G4 group mice injected with VEGF-H988 (50 mg/kg) intraperitoneally.

15 Figure 10 shows the mean blood concentration-time curve of G2&G4 groups mice injected with VEGF-H988 (50mg/kg) intraperitoneally .

Figure 11 shows the comparison of the efficacies of VEGF-H988 and the control drugs in the human colorectal cancer cell line HCT-116 xenograft tumor model.

20 Figure 12 shows the comparison of the efficacies of VEGF-H988 and the control drugs in the human colorectal cancer cell line HCT-116 xenograft tumor model.

DETAILED DESCRIPTION

25 Various aspects of the present invention relate to an isolated anti-VEGF antibody or antigen-binding fragment thereof, an antibody-drug conjugate comprising said antibody or antigen-binding fragment thereof, a nucleic acid and an expression vector encoding said antibody or antigen-binding fragment thereof, and a host cell
30 containing said nucleic acid or expression vector, a method for producing said anti-VEGF antibody or antigen-binding fragment

thereof, a pharmaceutical composition comprising said anti-VEGF antibody or antigen-binding fragment thereof, and a method of using said anti-VEGF antibody or antigen-binding fragment thereof for treating colorectal cancer.

5 *Definition*

Unless otherwise stated, all technical and scientific terms used herein have the meaning normally understood by a person skilled in the art to which the present invention belongs. For the purposes of the present invention, the following terms are defined to be consistent
10 with the meanings commonly understood in the art.

When used herein and in the appended claims, the singular forms "one", "a/an", "another" and "said" include the plural designation of the object unless the context clearly indicates otherwise.

The term "antibody" refers to an immunoglobulin molecule and
15 refers to any form of antibody that exhibits the desired biological activity. These include, but are not limited to, monoclonal antibodies (including full-length monoclonal antibodies), polyclonal antibodies and multispecific antibodies (e.g. bispecific antibodies), and even antibody fragments. Typically, full-length antibody structures
20 preferably comprise four polypeptide chains, two heavy (H) chains and two light (L) chains, typically interconnected by disulfide bonds. Each heavy chain contains a heavy chain variable region and a heavy chain constant region. Each light chain contains a light chain variable region and a light chain constant region. In addition to this typical
25 full-length antibody structure, the structure also includes other derivative forms.

Said heavy chain variable region and light chain variable region can be further subdivided into more conservative regions (called framework regions (FR)) and hypervariable regions (called complementarity
30 determining regions (CDR)) interspersed therewith.

The term "complementary determining region" (CDR, e.g. CDR1,

CDR2 and CDR3) refers to such amino acid residues in the variable region of an antibody whose presence is necessary for antigen binding. Each variable region typically has three CDR regions identified as CDR1, CDR2 and CDR3. Each complementary determining region may
5 contain amino acid residues from a "complementary determining region" as defined by Kabat (Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. 1991) and/or those residues from the "high-variable loop" (Chothia and Lesk; J MolBiol 196: 901-917
10 (1987)).

The term "framework" or "FR" residues are those residues within the variable region other than CDR residues as defined herein.

Each heavy chain variable region and light chain variable region typically contains 3 CDRs and up to 4 FRs, said CDRs and FRs being
15 arranged from the amino terminus to the carboxyl terminus in the following order, for example: FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4.

The complementary determining region (CDR) and the framework region (FR) of a given antibody can be identified using the
20 Kabat system (Kabat et al: Sequences of Proteins of Immunological Interest, 5th edition, US Department of Health and Human Services, PHS, NIH, NIH Publication No. 91- 3242, 1991).

The term "constant region" refers to such amino acid sequences in the light and heavy chains of an antibody that are not directly involved
25 in the binding of the antibody to the antigen but exhibit a variety of effector functions such as antibody-dependent cytotoxicity.

According to the antigenic differences of the amino acid sequence of its constant region, the heavy chain of an antibody can be classified into five classes: α , δ , ϵ , γ , and μ . When it forms a complete antibody
30 with the light chain, it can be classified into five classes: IgA, IgD, IgE, IgG and IgM, of which can be further classified into subclasses

(isotypes), such as IgG1, IgG2, IgG3, IgG4, IgA and IgA2. Based on the amino acid sequence of its constant domain, the light chain of an antibody can be classified into κ and λ .

An "antigen-binding fragment of an antibody" comprises a portion
5 of an intact antibody molecule that retains at least some of the binding specificity of the parent antibody and typically includes at least a portion of the antigen-binding region or variable region (e.g. one or more CDRs) of the parent antibody. Examples of antigen-binding fragments include, but are not limited to, Fv, Fab, Fab', Fab'-SH, F(ab')₂, Fd fragment, Fd'
10 fragment, single chain antibody molecules (e.g. scFv, di-scFv or tri-scFv, diabody or scFab), single domain antibodies.

The term "antibody fragment" refers to a non-intact antibody molecule that retains at least some of the biological properties of the parent antibody, including, but not limited to, an Fc fragment, in addition
15 to those described above as "antigen-binding fragments".

The term "antibody-drug conjugate" or "ADC" refers to a binding protein, such as an antibody or antigen-binding fragment thereof, that chemically linked to one or more of chemical drugs (also referred to as agents herein), which may optionally be a therapeutic agent or a
20 cytotoxic agent. In a preferred embodiment, an ADC includes an antibody, a cytotoxic or therapeutic drug, and a linker that enables the drug to be linked or conjugated to the antibody. ADCs usually have any value of 1 to 8 drugs conjugated to the antibody, including 2, 4, 6, or 8 drug-loading substances. Non-limiting examples of drugs that can be
25 included in the ADCs are mitotic inhibitors, anti-tumor antibiotics, immunomodulators, vectors for gene therapy, alkylating agents, anti-angiogenic agents, antimetabolites, boron-containing agents, chemotherapeutic protective agents, hormones, antihormonal agents, corticosteroids, photoactive therapeutic agents, oligonucleotides,
30 radionuclide agents, topoisomerase inhibitors, tyrosine kinase inhibitors and radiosensitizers.

The term "chimeric antibody" refers to an antibody in which a part of the heavy chain and/or light chain is derived from a specific source or species, and the remaining part is derived from a different source or species. The "chimeric antibody" may also be a functional fragment as defined above. "Humanized antibodies" are a subset of "chimeric antibodies."

The term "humanized antibody" or "humanized antigen-binding fragment" is defined herein as an antibody or antibody fragment that is: (i) derived from a non-human source (e.g., a transgenic mouse carrying a heterologous immune system) and based on a human germline sequence; or (ii) a chimeric antibody where the variable region is of non-human origin and the constant region is of human origin; or (iii) a CDR transplant where the CDR of the variable region is of non-human origin and one or more frame work regions of the variable region are of human origin and the constant region, if any, is of human origin. The aim of "humanization" is to eliminate the immunogenicity of antibodies of non-human origin in the human body, while retaining the greatest possible affinity. It is advantageous to select the human framework sequence that is most similar to the framework sequence of the non-human source antibody as the template for humanization. In some cases, it may be necessary to replace one or more amino acids in the human framework sequence with corresponding residues in the non-human construct to avoid loss of affinity.

The term "monoclonal antibody" refers to an antibody derived from a substantially homogeneous population of antibodies, i.e. every single antibody comprised in the population is identical except for possible mutations (e.g. natural mutations) which may be present in very small quantities. The term "monoclonal" therefore indicates the nature of the antibody in question, i.e. not a mixture of unrelated antibodies. In contrast to polyclonal antibody preparations, which usually comprise different antibodies against different epitopes, each monoclonal

antibody in a monoclonal antibody preparation is directed against a single epitope on the antigen. In addition to their specificity, monoclonal antibody preparations have the advantage that they are usually not contaminated by other antibodies. The term "monoclonal" should not be understood as requiring the production of said antibodies by any particular method.

The antibody "specifically binds" to a target antigen such as a tumor-associated peptide antigen target (in this case, PD-1), i.e. binds said antigen with sufficient affinity to enable said antibody to be used as a therapeutic agent, targeting a cell or tissue expressing said antigen, and does not significantly cross-react with other proteins, or does not significantly cross-react with proteins other than the homologues and variants of the target proteins mentioned above (e.g. mutant forms, splice variants, or protein hydrolysis truncated forms).

The term "binding affinity" refers to the strength of the sum of the non-covalent interactions between a molecule's individual binding sites and its binding partners. Unless otherwise stated, "binding affinity", when used herein, refers to the intrinsic binding affinity, which reflects a 1:1 interaction between members of a binding pair (e.g. antibody and antigen). As used herein, the term "KD" refers to the equilibrium dissociation constant of the antibody-antigen interaction. As used herein, the term " k_{on} " refers to the rate constant at which an antibody binds to an antigen. As used herein, the term " k_{off} " refers to the rate constant at which an antibody dissociates from an antibody/antigen complex. "KD", "binding rate constant k_{on} " and "dissociation rate constant k_{off} " are commonly used to describe the affinity between a molecule (e.g. an antibody) and its binding partner (e.g. an antigen). Affinity, i.e. the tight degree at which a ligand binds a particular protein. Binding affinity is influenced by non-covalent intermolecular interactions such as hydrogen bonding, electrostatic interactions, hydrophobic and van der Waals forces between two molecules. In addition, the binding affinity

between a ligand and its target molecule may be influenced by the presence of other molecules. Affinity can be analyzed by conventional methods known in the art, including the ELISA described herein.

5 The term "epitope" includes any protein determinant cluster that specifically binds to an antibody or T-cell receptor. Epitope determinant clusters typically consist of a molecule's chemically active surface groups (e.g. amino acid or sugar side chains, or a combination thereof) and often have specific three-dimensional structural features as well as specific charge characteristics.

10 The term "isolated" antibody is an antibody that has been identified and isolated from the components of the cell where the antibody expressed. Isolated antibodies include in situ antibodies inside of recombinant cells, where at least one component in natural environment of said antibody is absent. However, usually, the isolated antibody is
15 prepared through at least one purification step.

"sequence identity" between two polypeptides or nucleic acid sequences indicates the number of residues that are identical between said sequences as a percentage of the total number of residues, and is calculated based on the size of the smaller of the compared
20 molecules. When calculating the percentage identity, the sequences being aligned are matched in such a way as to produce a maximum match between the sequences, with the gaps in the match (if present) being resolved by a specific algorithm. Preferred computer program methods for determining identity between two sequences include, but
25 are not limited to, GCG program packages including GAP, BLASTP, BLASTN and FASTA (Altschul et al., 1990, J. Mol. Biol. 215: 403-410). The above procedures are publicly available from the International Center for Biotechnology Information (NCBI) and other sources. The well-known Smith Waterman algorithm can also be used to determine
30 identity.

The term "Fc receptor" or "FcR" refers to a receptor that binds to the Fc region of an antibody. Human FcRs of natural sequence are preferred, and preferably receptors that bind to IgG antibodies (gamma receptors), which include the FcγRI, FcγRII and FcγRIII isoforms, as well as variants of these receptors. All other FcRs are included in the term "FcR". The term also includes the neonatal receptor (FcRn), which is responsible for the transport of maternal IgG to the fetus (Guyer et al, Journal of Immunology 117: 587 (1976) and Kim et al, Journal of Immunology 24: 249 (1994)).

The term "neonatal Fc receptor", abbreviated as "FcRn", binds to the Fc region of IgG antibodies. The neonatal Fc receptor (FcRn) plays an important role in the metabolic fate of IgG-like antibodies *in vivo*. FcRn functions to rescue IgG from the lysosomal degradation pathway, thereby reducing its clearance in serum and lengthening its half-life. Therefore, the *in vitro* FcRn binding properties/characteristics of IgG are indicative of its *in vivo* pharmacokinetic properties in the circulation.

The term "effector function" refers to those biological activities attributable to the Fc region of an antibody, which vary from isotype to isotype. Examples of antibody effector functions include C1q binding and complement-dependent cytotoxicity (CDC), Fc receptor binding, antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), cytokine secretion, immune complex-mediated uptake of antigen by antigen-presenting cells, cell surface receptors down-regulation (e.g. B-cell receptors) and B-cell activation.

The term "effector cell" refers to a cell that expresses one or more FcRs and performs effector functions. In one aspect, said effector cells express at least FcγRIII and perform ADCC effector functions. Examples of human cells that mediate ADCC include peripheral blood mononuclear cells (PBMCs), natural killer (NK) cells, monocytes, cytotoxic T cells and neutrophils. Effector cells can be isolated from

natural sources, for example, blood. Effector cells are usually lymphocytes associated with effector phase and function to produce cytokines (helper T cells), kill cells infected by pathogens (cytotoxic T cells) or secrete antibodies (differentiated B cells).

5 "Immune cells" include cells that have a haematopoietic origin and play a role in the immune response. Immune cells include: lymphocytes, such as B cells and T cells; natural killer cells; and myeloid cells, such as monocytes, macrophages, eosinophils, mast cells, basophils and granulocytes.

10 "Antibody-dependent cell-mediated cytotoxicity" or "ADCC" refers to a form of cytotoxicity in which secreted Ig binds to Fcγ receptors presented on certain cytotoxic cells (e.g. NK cells, neutrophils and macrophages) allows these cytotoxic effector cells to specifically bind to target cells bearing antigens and subsequently kill
15 said target cells using, for example, a cytotoxin. To assess the ADCC activity of the target antibody, *in vitro* ADCC assays can be performed, such as the *in vitro* ADCC assays documented in US Patent No. 5,500,362 or 5,821,337 or US Patent No. 6,737,056 (Presta). Useful effector cells for use in such assays include PBMCs and NK cells.

20 "Complement-dependent cytotoxicity" or "CDC" refers to the lysis of target cells in the presence of complement. The classic pathway for complement activation is initiated by binding the first component of the complement system (C1q) to an antibody (of the appropriate subclass) that binds to its corresponding antigen. To assess
25 complement activation, a CDC assay can be performed, such as the CDC assay recited in Gazzano-Santoro et al., J. Immunol Methods 202: 163 (1996). For example in US Patent No. 6,194,551 B1 and WO1999/51642, there described polypeptide variants having altered amino acid sequences of the Fc region (polypeptides having a variant
30 Fc region) and polypeptide variants having enhanced or reduced C1q binding.

Amino acid sequence and nucleotide sequence of the antibody of the present invention

The present invention used recombinant human VEGF165 protein to immunize rabbit, and then obtained the antibody clones VEGF-R859, VEGF-R988, VEGF-R613 and VEGF-R812 that specifically bind to recombinant human VEGF165 protein by phage display library screening. The nucleotide sequence encoding the heavy and light chain variable regions of the VEGFR2-MK19 scFv antibody was then inserted by PCR into pSTEP2 vector harboring nucleotide sequence encoding the rabbit IgG1 constant region or the rabbit kappa constant region, and cultured for expression. The high purity antibodies were purified using a protein A purification column. ELISA showed that said rabbit antibody was able to block the binding of VEGF165 protein to VEGFR2 protein, and VEGF-R988 and VEGF-R613 could effectively reduce the ability of VEGF165 to promote HUVEC proliferation, and VEGF-R988 showed a higher maximum inhibition rate.

Then, using the classic method for humanized CDR transplantation, the human antibody light chain or heavy chain variable region whose sequence is closer to the sequence of rabbit light chain or heavy chain variable region was elected as the template, the humanized light chain variable region (VL) and heavy chain variable region (VH) sequences were obtained by inserting each of the three CDRs (Table 1) of the rabbit antibody light chain or heavy chain into the variable regions of said human antibody. As the key sites of the rabbit framework region are essential for maintaining the stability of the CDR activity, the key sites were reverse-mutated to the corresponding sequence of rabbit antibody. VEGF-H988-10 light chain/heavy chain expression vector was obtained by whole gene synthesis, transfected into HEK-293 cells and cultured for expression, and the culture supernatant was purified using a protein A purification column to obtain high purity antibodies. To improve the affinity of VEGF-H988-10, SDM libraries of CDR regions of heavy and

light chain variable regions (including LCDR1, LCDR3, HCDR2 and HCDR3) were constructed, and the four mutant libraries were constructed in scFv form and were cloned into phage vectors as svFv-gIII fusion protein; for each CDR, the CDR clones having optimal binding ability to soluble antigen VEGF were screened, and finally the antibody VEGF-H988 having optimized CDR affinity and stability was obtained.

Nucleic acids of the present invention

The present invention also relates to nucleic acid molecules encoding antibodies or portions thereof of the present invention. The sequences of these nucleic acid molecules include, but are not limited to, SEQ ID NOs: 11, 19-20, 23-24, 43-51 and 53-54.

The nucleic acid molecules of the present invention are not limited to the sequences disclosed herein, but also include variants thereof. Variants in the present invention may be described with reference to their physical properties in hybridization. It will be recognized by those of skill in the art that using nucleic acid hybridization techniques, nucleic acids can be used to identify their complements as well as their equivalents or homologues. It will also be recognized that hybridization can occur at less than 100% complementarity. However, given the appropriate choice of conditions, hybridization techniques can be used to distinguish said DNA sequences based on the structural relevance of the DNA sequence to a particular probe. For guidance on such conditions see Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Ed. Cold Spring Harbor Press, Cold Spring Harbor, N. Y., 1989 and Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Sedman, J. G., Smith, J. A., &Struhl, K. eds. (1995). Current Protocols in Molecular Biology. New York: John Wiley and Sons.

Recombinant vectors and expression

The present invention also provides recombinant constructs comprising one or more nucleotide sequences of the present invention. The recombinant construct of the present invention is constructed by

inserting the nucleic acid molecule encoding the antibody of the present invention into a vector such as a plasmid, phagemid, phage or viral vector.

The antibodies provided herein can be prepared by recombinantly
5 expressing nucleotide sequences encoding light and heavy chains or
portions thereof in a host cell. In order to recombinantly express the
antibody, the host cell may be transfected with one or more recombinant
expression vectors carrying nucleotide sequences encoding the light
10 and/or heavy chains or portions thereof, so that said light and heavy
chains are expressed in said host cell. Standard recombinant DNA
methodologies are used to prepare and/or obtain nucleic acids encoding
heavy and light chains, to incorporate these nucleic acids into
recombinant expression vectors and to introduce said vectors into host
cells, e.g. Sambrook, Fritsch and Maniatis (eds.), Molecular Cloning; A
15 Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., (1989),
Ausubel, F. M. et al. (eds.) Current Protocols in Molecular Biology,
Greene Publishing Associates, (1989) and those documented in U.S.
Patent No. 4,816,397 by Boss et al.

Suitable host cells are prokaryotic and eukaryotic cells. Examples
20 of prokaryotic host cells are bacteria and examples of eukaryotic host
cells are yeast, insect or mammalian cells. It should be understood that
the design of an expression vector including the selection of a regulatory
sequence is determined by a number of factors, such as the choice of
host cell, the level of expression of the desired protein and whether the
25 expression is constitutive or inducible.

Bacterial expression

By inserting a structural DNA sequence encoding the desired
antibody together with appropriate translation initiation and termination
signals and a functional promoters into an operable reading frame, an
30 expression vector for use in bacteria is constructed. The vector will
contain one or more phenotypic selection markers and an origin of

replication to ensure the maintenance of the vector and provide amplification in the host as needed. Suitable prokaryotic hosts for transformation include multiple species of *E. coli*, *Bacillus subtilis*, *Salmonella typhimurium*, as well as *Pseudomonas*, *Streptomyces* and
 5 *Staphylococcus*.

The bacterial vector may be, for example, phage-, plasmid- or phagemid-based. These vectors may contain selection markers and bacterial replication origins, which are derived from commercially available plasmids that usually contain elements of the well-known
 10 cloning vector pBR322 (ATCC 37017). After transforming an appropriate host strain and growing the host strain to an appropriate cell density, the selected promoter is de-repressed/induced by an appropriate method (for example, temperature change or chemical induction), and the cells are cultured for an additional time. The cells are usually
 15 harvested by centrifugation, disrupted by physical or chemical methods, and the resulting crude extract is retained for further purification.

In a bacterial system, a variety of expression vectors can be advantageously selected according to the intended use of the expressed protein. For example, when a large number of such proteins are to be
 20 produced for antibody production or for peptide library screening, for example, a vector that directs high-level expression of a fusion protein product to be easily purified may be required.

Mammalian Expression and Purification

Preferred regulatory sequences for expression in mammalian host
 25 cells include viral elements that direct high-level protein expression in mammalian cells, such as promoters and/or enhancers derived from cytomegalovirus (CMV) (e.g., CMV promoter/enhancer), promoters and/or enhancers of simian virus 40 (SV40) (e.g. SV40 promoter/enhancer), promoters and/or enhancers of adenovirus (e.g.
 30 adenovirus major late promoter (AdMLP)) and promoters and/or enhancers of polyoma virus. For a further description of viral regulatory

elements and their sequences, see, for example, U.S. 5,168,062 by Stinski, U.S. 4,510,245 by Bell et al., and U.S. 4,968,615 by Schaffner et al. The recombinant expression vector may also include an origin of replication and a selection marker (see, for example, U.S. 4,399,216, U.S. 4,634,665 and U.S. 5,179,017 by Axel et al). Suitable selection markers include genes that confer resistance to drugs such as G418, hygromycin, or methotrexate to host cells into which the vector has been introduced. For example, the dihydrofolate reductase (DHFR) gene confers resistance to methotrexate, while the *neo* gene confers resistance to G418.

The transfection of the expression vector into host cells can be performed using standard techniques such as electroporation, calcium phosphate precipitation, and DEAE-dextran transfection.

Suitable mammalian host cells for expressing the antibodies provided herein include Chinese Hamster Ovary (CHO cells) [including dhfr-CHO cells, as described in Urlaub and Chasin, (1980) Proc. Natl. Acad. Sci. USA 77:4216-4220, DHFR selection markers are employed, as described in, for example, R.J. Kaufman and P.A. Sharp (1982) Mol. Biol. 159:601-621], NSO myeloma cells, COS cells, and SP2 cells.

The antibodies of the present invention can be recovered and purified from recombinant cell culture by known methods, including but not limited to, ammonium sulfate or ethanol precipitation, acid extraction, protein A affinity chromatography, protein G affinity chromatography, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxyapatite chromatography, and lectin chromatography. High performance liquid chromatography ("HPLC") can be used for purification as well. See, for example, Colligan, Current Protocols in Immunology, or Current Protocols in Protein Science, John Wiley & Sons, NY, N.Y., (19972001), for example, Chapters 1, 4, 6, 8, 9, and 10.

Characteristics and functions of the antibodies of the present invention

Characteristic analysis and function analysis of the humanized antibody VEGF-H988 of the present invention were performed.

- 5 The analyses showed that the antibody of the present invention has the following advantages: (1) The ability of VEGF-H988 to bind VEGF165 protein is slightly better than that of Avastin; (2) The binding affinity of VEGF165-H988 to recombinant human VEGF165 protein is slightly higher than that of Avastin, which is about 1.5 times that of Avastin; (3)
- 10 VEGF165-H988 specifically binds to recombinant human VEGF165 protein and cross-binds to recombinant mouse mVEGF164 protein; (4) antibody VEGF-H988 can effectively inhibit the binding of VEGFR2 protein to VEGF165 protein, and its inhibitory ability is weaker than EYLEA but better than Avastin; (5) antibody VEGF-H988 can effectively
- 15 reduce the ability of VEGF165 to promote HUVEC proliferation; (6) antibody VEGF-H988 has a stronger neutralizing effect than Avastin in the case of different isoforms of VEGF (VEGF165, VEGFC, VEGFD) acting simultaneously on HUVEC cells; (7) Repeated drug administration toxicity testing in mice showed that no significant drug-related toxic reactions were
- 20 observed with antibody VEGF-H988 ; and (8) Tumor suppression test of HCT-116 xenograft tumor model showed that VEGF-H988 has a better effect on tumor inhibition than Avastin.

Uses

- 25 The antibodies of the present invention can be used to treat colorectal cancer. The antibody of the present invention can also be used to prepare medicines for the treatment of said disorders.

Pharmaceutical compositions

Antibodies of the present invention may be prepared with at least

one other agent (e.g. a stable compound) to form pharmaceutical compositions comprising an antibody of the present invention and one or more pharmaceutically acceptable carriers, diluents or excipients. Optionally, the pharmaceutical compositions may contain additional
5 therapeutic agents.

Kits

The present invention also relates to a pharmaceutical package and a kit comprising one or more containers, said containers contains the foregoing pharmaceutical compositions of the present invention.
10 Accompanied with such containers may be specifications in the form prescribed by the governmental agency governing the manufacture, use or distribution of the drug or biological product, which reflect approval for human administration by the agency in which said product is manufactured, used or distributed.

Preparation and storage

The pharmaceutical compositions of the present invention can be prepared in a manner known in the art, for example by conventional mixing, dissolution, granulation, pastille preparation, grinding, emulsification, encapsulation, embedding or lyophilization methods.

20 Having already prepared pharmaceutical compositions comprising compounds of the present invention formulated in an acceptable carrier, they may be placed in appropriate containers and labeled for the treatment of the condition indicated. Such labeling would include the amount, frequency and administration routes of the drug.

Combinations

25 The pharmaceutical compositions comprising the antibodies of the present invention described above are also combined with one or more other therapeutic agents, such as antineoplastic agents, wherein the resulting combination does not cause unacceptable adverse effects.

30

The following examples facilitate a better understanding of the

present invention, but do not intend to limit the present invention. The experimental methods in the following examples, unless otherwise specified, are all conventional methods. The experimental materials used in the following examples, unless otherwise specified, were purchased from conventional biochemical reagent distributors.

EXAMPLES

Example 1: Screening of rabbit antibodies that block the binding of VEGF165 to VEGFR1/VEGFR2 using antibody phage display library

1.1 Immunization of rabbits

Recombinant human VEGF165 protein (from Sino Biological, Inc, Cat. 11066-HNAH) was used to immunize rabbits. The amino acid sequence of the extracellular region Met1-Arg191 of the human VEGF165 protein (UniProt P15692-4) is SEQ ID NO: 1.

The detailed method was: The recombinant human VEGF165 protein was mixed with Freund's adjuvant, the rabbits were subcutaneously immunized with the mixture for 4 times at intervals of 3 weeks, 2 weeks and 2 weeks respectively, at a dose of 500 µg each time. Since the fourth immunization, blood was collected 4 days after immunization via the medial canthal plexus of the eye. The serum titer of rabbit anti-VEGF165 was measured by ELISA using coated recombinant human VEGF165 protein. The titer of the serum from the fifth immunization reached 1:250000, and the rabbits were boosted intravenously with 25 µg recombinant human VEGF165 protein 9 weeks after the fifth immunization. 7 days later, the mice were executed and the spleen tissue was removed and frozen in liquid nitrogen.

1.2 Screening of antibody phage-display library

RNA was extracted from rabbit spleen tissue using TriPure™ Isolation Reagent (from Roche, Cat. No.11 667 165 001), and cDNAs were

obtained by reverse transcription of RNA using a reverse transcription kit (from Invitrogen Cat.No.18080-051). 10 pairs of primers were designed to amplify the sequence of the light chain variable region of the rabbit antibody and 4 pairs of primers were designed to amplify the sequence of the heavy chain variable region (Barbas C F et al., CSHL Press. 2004). The sequences encoding the light and heavy chain variable regions of the rabbit antibody were assembled into the nucleotide sequence encoding scFv by overlapping extension PCR, the light and heavy chain variable regions were linked (Jones S T et al., Bio/technology. 1991; 9(1): 88) by the following linker:

TCTAGTGGTGGCGGTGGTTCGGGCGGTGGTGGAGGTGGT
AGTTCTAGATCTTCC (SSGGGSGGGGGGSSRSS) (SEQ ID NO: 2);

Then enzymatically ligated into the phage vector pComb3x (from Sino Biological, Inc.) by restriction endonuclease *Sfi* I (from Fermentas), and was electrotransformed into the competent X-Blue to construct the rabbit phage-display scFv antibody library. The recombinant human VEGF165 protein was coated on an ELISA plate, and a phage library enriched with anti-VEGF165 positive antibodies was screened according to the process of phage antibody panning process (O'Brien, PM, & Aitken, R. (Eds.), Springer Science & Business Media. 2002; ISBN: 9780896037113). Single colony phages were selected from the enriched library for expression, and their binding to recombinant human VEGF165 protein was detected by ELISA. The scFv antibody clones that specifically bind to recombinant human VEGF165 were selected and were sent to a sequencing service company for sequencing to obtain the nucleotide sequences of the antibodies, wherein several scFv antibody clones were derived into VEGF-R859, VEGF-R988, VEGF-R613, VEGF-R812 by the method described in Example 1.3. The nucleotide sequences of their scFV antibody clones are SEQ ID NO: 3. SEQ ID NO: 4, SEQ ID NO: 5, and SEQ ID NO: 6.

1.3 Production of rabbit antibodies targeting VEGF165

Taking VEGF-R988 as an example, the nucleotide sequence encoding the heavy chain variable region of the scFv antibody of VEGF-R988 was PCR amplified and inserted into the *Sca* I + *Kpn* I (Fermentas) digested pSTEP2 vector harboring nucleotide sequences encoding the heavy chain signal peptide (SEQ ID NO: 45) and rabbit IgG1 constant region (SEQ ID NO: 9) by in-fusion method, thus the heavy chain (SEQ ID NO:53) expression vector was obtained. The nucleotide sequence encoding the light chain variable region of the scFv antibody of VEGF-R988 was PCR amplified and inserted into the *Sca* I + *Bam*H I (Fermentas) digested pSTEP2 vector harboring nucleotide sequences encoding the light chain signal peptide (SEQ ID NO: 46) and rabbit kappa constant region (SEQ ID NO: 10) by in-fusion method, thus the light chain (SEQ ID NO: 54) expression vector was obtained. The recombinant plasmids were extracted, and transfected into HEK-293 cells and cultured for expression for 7 days, and the culture supernatant was purified by a protein A purification column to obtain high-purity antibodies.

Primers for amplifying the heavy chain variable region:

F1	ACCAGGGTGCTGAGTCAGTCGGTGGAGGAGTCC
R1	TGTGACCAGGGTACCTGGGCCCCA

Primers for amplifying the light chain variable region:

F2	ACAGGAGTGCATAGTGAGCTCGATCTGACCCAG AC
R 2	GGTGCAACTGGATCCCCTTTGACGACCACCTCG GT

1.4 Function analysis of rabbit antibodies targeting VEGF165

1.4.1 Rabbit antibody blocks VEGF165 from binding to VEGFR2-his VEGF165 protein (from SinoBiological, Inc.) at a concentration of

1 $\mu\text{g/mL}$ was coated on a 96-well plate in 100 μL /well overnight at 4°C. The plate was washed the next day and blocked at room temperature for 1 h. 100 μL of 5 $\mu\text{g/mL}$ VEGFR2-biotin protein (from SinoBiological, Inc.) and said rabbit antibodies targeting VEGF165 at different
 5 concentrations were added and co-incubated. The plate was washed to remove unbound antibodies, incubated with Streptavidin/HRP (from Beijing ZSGB-Bio Co., Ltd.) and then repeatedly washed, and the chromogenic substrate solution was added for color development. OD₄₅₀ was measured after the color development was ended. Taking the
 10 concentration of the rabbit antibody targeting VEGF165 as the horizontal coordinate and the inhibition rate PI% as the vertical coordinate, the graphPad Prism 6.0 software was used for data analysis and generating a curve chart. Inhibition rate (%) = $(\text{OD}_{\text{blank}} - \text{OD}_{\text{sample}}) / \text{OD}_{\text{blank}} \times 100\%$, where OD_{blank} indicates the OD value of the wells with
 15 only VEGFR2-biotin added but no rabbit antibody, and OD_{sample} indicates the OD value of the wells with both VEGFR2-biotin and rabbit antibodies added.

As shown in Figure 1, VEGFR2 protein can effectively bind to the coated VEGF165 protein, and the rabbit antibodies of VEGF-R859,
 20 VEGF-R988, VEGF-R613, VEGF-R812 can effectively inhibit the binding of VEGFR165 protein to VEGFR2 protein.

Antibody inhibits the proliferative effect of VEGF 165 on umbilical vein endothelial cells

1.4.2 Rabbit antibody inhibits the proliferation of HUVEC

25 The effect of said rabbit antibodies neutralizing the VEGF 165-induced umbilical vein endothelial cells proliferation was detected by using the WST-8 method. Human umbilical vein endothelial cells HUVEC were inoculated into a 96-well plate at 4×10^3 cells/well, cultured in M199 medium containing 10% FBS and 5% L-Gln for 4 h, and then
 30 different concentrations of rabbit antibodies were added in 50 μL /well, then VEGF-165 at a final concentration of 10 ng/mL was added in 10 μL /well,

the 96-well plate was incubated in a 37°C, 5% CO₂ cell incubator for 3 days, and the blank well B (no cells), negative control M (cells inoculated, no antibody sample, VEGF-165 added) and M' (cells inoculated, no antibody sample and no VEGF-165) were used. After incubation, 10 μ L/well of WST-8 chromogenic solution was added, and the 96-well plate was incubated in CO₂ incubator for color development, OD₄₅₀ and OD₆₃₀ were measured with a microplate reader after the color development was stabilized. For each well, the reading value was (OD₄₅₀ – OD₆₃₀), and the neutralization rate of the antibody was calculated as the OD value for each group was defined as the reading value of the group minus the reading value of blank well B, the neutralization rate% = (OD value of negative control M – OD value of sample) / (OD value of negative control M – OD value of M') $\times$ 100%. The standard curve was calculated using the automatic analysis function of the statistical software GraphPad Prism, taking the antibody sample concentration as the horizontal coordinate and the neutralization rate as the vertical coordinate, and the four-parameter logistic regression equation was used to fit the standard “S” curve to calculate the half maximal effective concentration (EC₅₀) of the antibody sample.

The results shown in Figure 2 demonstrate that VEGF-R988 and VEGF-R613 effectively reduce the ability of VEGF165 to promoting HUVEC proliferation, and VEGF-R988 shows a higher maximum inhibition rate, VEGF-R812 has basically no inhibitory ability, while VEGF-R859 has no inhibitory ability at all.

25

Example 2: Humanization, modification and production of rabbit antibody VEGF-R988

On the ground of the results of the functional analysis of rabbit antibody in **Example 1**, VEGF-R988 was selected for humanization and production.

30

2.1 Determination of the CDRs of the light and heavy chains of

rabbit antibody VEGF-R988

Based on the nucleotide sequence of the VEGF-R988 scFv antibody determined in **Example 1.2**, the amino acid sequences of the heavy chain and light chain variable regions of the VEGF-R988 scFv were deduced, see SEQ ID NO: 11/12.

Refer to the Kabat index and IMGT numbering systems, the amino acid sequence of each of the three CDRs of the light and heavy chains of the rabbit antibody VEGF-R988-scFv were determined, see **Table 1**. The aforementioned respective three CDRs of the light chain and the heavy chain were transplanted into the humanized antibody VEGF-R988-scFv in the subsequent steps, see **Example 2.2**.

Table 1: CDR sequences of VEGF-R988 light chain and heavy chains

Name	Sequences
LCDR1	QSSQTIYANRRLA (SEQ ID NO:13)
LCDR2	GASTLAS (SEQ ID NO:14)
LCDR3	AGYKSYDGDDVG (SEQ ID NO:15)
HCDR1	GIDLSSYAISWV (SEQ ID NO:16)
HCDR2	YIWNAGNTYYASWAKG (SEQ ID NO:17)
HCDR3	ARGTLGDYNGMDP (SEQ ID NO:18)

2.2 CDR transplantation of the rabbit antibody VEGF-R988

The humanization of the rabbit antibody was performed using the classic humanization method of CDR transplantation. The human antibody light or heavy chain variable region, whose sequence is closer to the sequence of rabbit light or heavy chain variable region, was elected as the template, and each of three CDRs (Table 1) from the rabbit

light or heavy chain were inserted into the variable regions of the human antibody to obtain the humanized light chain variable region (VL) or heavy chain variable region (VH) sequences respectively. The human template for the light chain variable region of VEGF-R988 is IGKV1-27*01, which is 65.30% homologous to the light chain of VEGF-R988, and the human template for the heavy chain variable region is IGHV4-4*08, which is 53.20% homologous to the heavy chain of VEGF-R988.

2.3 Reverse-mutations at the framework region of the humanized variable region

As some key amino acids in the rabbit-derived framework region are essential to maintain the CDR activity, the key amino acids were reverse-mutated to the corresponding rabbit antibody amino acid sequences, the following sites were reversely mutated: in the light chain, Position 1 was reversely mutated to E, Position 2 was reversely mutated to L, Position 4 was reversely mutated to L, and Position 63 was reversely mutated to K; while in the heavy chain, Position 3 was reversely mutated to V, Position 37 was reversely mutated to V, Position 47 was reversely mutated to Y, Position 78 was reversely mutated to V, Position 79 was reversely mutated to D, and Position 91 was reversely mutated to F; all the above sites were numbered by reference to the Kabat numbering scheme. The humanized antibody VEGF-H988-10 was obtained by CDR humanized transplantation and framework region reverse-mutations.

2.4 Production of humanized monoclonal antibody VEGF-H988-10 and CDR affinity modification

VEGF-H988-10 heavy chain variable region (SEQ ID NO: 23) was obtained by the whole gene synthesis method, and then inserted, by infusion method, into *Sca* I + *Nhe* I (Fermentas) digested pSTEP2 vector harboring the nucleotide sequence encoding the heavy chain signal peptide (SEQ ID NO: 45) and the nucleotide sequence encoding the

human IgG1 constant region (SEQ ID NO: 49), to obtain the VEGF-H988-10 heavy chain (SEQ ID NO:19) expression vector. VEGF-H988-10 light chain variable region (SEQ ID NO: 24) was obtained by the whole gene synthesis method, and then inserted, by in-fusion method,
 5 into *Sca* I + *Bsi*W I (Fermentas) digested pSTEP2 vector harboring the nucleotide sequence encoding the light chain signal peptide (SEQ ID NO: 46) and the nucleotide sequence encoding the human kappa constant region (SEQ ID NO: 50) to obtain the VEGF-H988-10 light chain (SEQ ID NO: 20) expression vector. Plasmids were extracted and
 10 co-transfected into HEK-293 cells, the cells were cultured for 7 days. The culture supernatant was purified with a protein A purification column to obtain high-purity antibodies.

Primers for whole gene synthesis of the heavy chain variable region

15

F3	CCACAGGAGTGCATAGTGAAGTCCAACTTACCCAGAGCCCAT CCTCCCTG
R3	CCTGTCTCCCACAGAGGCAGACAGGGAGGATGG
F4	TCTGTGGGAGACAGGGTGACCATCACTTGTCAG
R4	GGCATAGATGGTCTGGCTGGACTGACAAGTGAT
F5	CAGACCATCTATGCCAACAGGAGACTGG
R5	TTCTGTTGATACCAAGCCAGTCTCCTGT
F6	TTGGTATCAACAGAAGCCTGGCAAGGTG
R6	AAATCAGCAGTTTTTGGCACCTTGCCAGG
F7	CAAACTGCTGATTTATGGAGCCAGCAC
R7	CACTCCAGATGCCAGGGTGCTGGCTCCA
F8	CTGGCATCTGGAGTGCCAAGCAGGTTCAAGGGC
R8	GAAGTCTGTGCCAGAGCCAGAGCCCTTGAACCT
F9	TCTGGCACAGACTTCACCCTGACCATCTCCTCC
R9	AGCCACATCCTCAGGTTGGAGGGAGGAGATGGT
F10	CCTGAGGATGTGGCTACCTACTACTGTGCTGGC
R10	ATCTCCATCATAGGACTTGTAGCCAGCACAGTA
F11	TCCTATGATGGAGATGATGTGGGCTTTGGAGGA

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R11	GGTGCAGCCACCGTACGCTTAATCTCCACCTTGGTGCCTCCTC CAAAGCC
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Primers for whole gene synthesis of the light chain variable region

F12	GCTACCAGGGTGCTGAGTCAGTCTGTCCAGGAGTCTGGACCTG GACTGGTG
R12	GGACAGGGTCTCAGATGGCTTCACCAGTCCAGG
F13	TCTGAGACCCTGTCCCTGACTTGTACTGTGTCT
R13	ATAGGAGGACAGGTCAATGCCAGACACAGTACA
F14	GACCTGTCCTCCTATGCCATCTCCTGGGTGA
R14	CCCTTGCCAGGAGGTTGTCTCACCAGGAGA
F15	ACCTCCTGGCAAGGGATTGGAATACATTGGC
R15	TGCCAGCATTCCAGATGTAGCCAATGTATTC
F16	TCTGGAATGCTGGCAACACCTACTATGCCTC
R16	CACCCTGCCCTTAGCCCAGGAGGCATAGTAG
F17	GCTAAGGGCAGGGTGACCATCTCTGTGGACACC
R17	CAGGTCCACCTGGTTCTTGCTGGTGTCCACAGA
F18	AACCAGGTGGACCTGAAACTGTCCTCTGTGACA
R18	GTAGACTGCTGTGTCTGCTGCTGTCACAGAGGA
F19	GACACAGCAGTCTACTTCTGTGCCAGGGGCACC
R19	CATCCCATTGTAGTCTCCCAGGGTGCCCCTGGC
F20	GACTACAATGGGATGGACCCATGGGGACCTGGC
R20	GGGCCCTTGGTGCTAGCGCTGGACACTGTCACCAGGGTGCCAGGTC CCCA

5

To improve the affinity of VEGF-H988-10, SDM libraries of CDR regions of heavy and light chain variable regions (including three saturated mutation libraries of LCDR1, LCDR3, and HCDR2) were constructed; Meanwhile, to improve the chemical stability of the antibody, the amino acid residues capable of undergoing deamidation or isomerization should

10

be modified to another amino acid residue. The deamidation of asparagine can occur in, such as NG, NS, NA, NT, etc., leading to the generation of isoaspartic residues, which affects the stability or biological function of the antibody. The VEGF-H988 variable region HCDR3 has (a) deamidation-susceptible site(s), thus SDM libraries were constructed to improve the chemical stability and biological function of the antibody. The above four mutant libraries were constructed in scFv form and were cloned into phage vector as scFv- gIII fusion protein; for each CDR, the CDR clones having optimal binding ability to soluble antigen VEGF were screened, and finally the antibody VEGF-H988 having optimized CDR affinity and stability was obtained. The sequences of VEGF-H988 light and heavy chain CDRs are shown in **Table 2**.

Table 2: CDR sequences of VEGF-H988 light chain and heavy chains

Name	Sequences
LCDR1	QSSKFLWQGRRLA (SEQ ID NO:27)
LCDR2	GASTLAS (SEQ ID NO:28)
LCDR3	AGYKSYDGDVVG (SEQ ID NO:29)
HCDR1	GIDLSSYAIS (SEQ ID NO:30)
HCDR2	YIWNDLFTYYASWAKG (SEQ ID NO:31)
HCDR3	ARGTLGDYGGMDP (SEQ ID NO:32)

2.5 Production of humanized monoclonal antibody VEGF-H988

The nucleotide sequence (SEQ ID NO: 44) encoding the aforementioned antibody VEGF-H988 light chain and the signal peptide, which contains the following nucleotide sequences encoding light chain signal peptide (SEQ ID NO: 46), the humanized antibody light chain variable region (SEQ ID NO: 48) and the human antibody kappa light chain constant region (SEQ ID NO: 50) connected in order, was PCR

amplified and inserted into the self-developed pGS vector (*Kpn* I+*Xba* I) by in-fusion method, and the correct plasmids were verified by sequencing. The nucleotide sequence (SEQ ID NO: 43) encoding the aforementioned antibody VEGF-H988 heavy chain and the signal peptide, which contains the following nucleotide sequences encoding heavy chain signal peptide (SEQ ID NO: 45), the humanized antibody heavy chain variable region (SEQ ID NO: 47) and the human antibody kappa heavy chain constant region (SEQ ID NO: 49) connected in order, was PCR amplified and inserted into pGS vector (*Nhe* I+*Not* I) which had been verified to contain the light chain correctly by in-fusion method, and the correct vectors expressing both light and heavy chains of VEGF-R988 were verified by sequencing. These expression vectors are eukaryotic expression vectors containing the GS genes as the selection marker and the expression elements of the antibody light and heavy chains. These expression vectors were transfected into CHO-K1-GS-deficient cells and VEGF-H988 high expression cell lines were obtained by MSX screening. The clones with high antibody expression were selected by ELISA assay, and the high expression cell lines were selected by taking into account both the cell growth status and the key quality characteristics for antibody drugs. A serum-free suspension culture was used to culture the VEGF-H988 producing CHO cell line to obtain high purity and quality VEGF-H988 antibodies.

Example 3: Characteristic analysis of humanized antibody VEGF-H988

3.1 Characteristic analysis of humanized antibody VEGF-H988 binding to VEGF165

3.1.1 Humanized antibody VEGF-H988 specifically binds to VEGF165

Recombinant human VEGF165 protein (from SinoBiological, Inc.) in different concentrations (0.15ng/mL, 0.46 ng/mL, 1.37 ng/mL,

4.12ng/mL, 12.35 ng/mL, 37.04 ng/mL, 111.11 ng/mL, 333.33 ng/mL, 1000 ng/mL and 3000 ng/mL) was coated on a 96-well plate overnight at 4°C in 100µL/well. The plate was washed the next day and blocked at room temperature for 1 h. After incubation with 100 µL of 1 µg/mL of VEGF165-H988, Avastin (from Roche) or negative control antibody H7N9-R1 respectively, the plate was washed to remove unbound antibodies, then incubated with goat F(ab')₂ anti-human IgG F(ab')₂/HRP (from Jackson ImmunoResearch Laboratories, Inc.) and washed repeatedly, and the chromogenic substrate solution was added for color development. OD₄₅₀ was measured after the color development was ended. Taking the concentration of recombinant human VEGF165 protein as the horizontal coordinate and the OD₄₅₀ value as the vertical coordinate, the graphPad Prism 6.0 software was used to fit an “S” curve chart and the binding of the antibody to recombinant human VEGF165 protein was analyzed.

The results shown in Figure 3 demonstrate that the EC₅₀ value of humanized molecule VEGF165-H988 specifically binding to recombinant human VEGF165 is 2.42 ng/mL, R² = 0.999; the EC₅₀ value of Avastin binding to recombinant human VEGF165 is 2.77 ng/mL, R² = 1.000. This indicates that the ability of VEGF165-H988 binding to recombinant human VEGF165 protein is slightly better than that of Avastin. The negative control antibody H7N9-R1 has no binding ability to recombinant human VEGF165 protein.

3.1.2 Assay of the binding affinity of humanized antibody VEGF-H988 to recombinant VEGF165 protein

The affinities of VEGF165-H988 and Avastin (from Roche) were measured at multiple concentrations using streptavidin-coated Sensor and immobilized biotin-labeled VEGF165 protein.

The recombinant human VEGF165 protein was first labeled with biotin in a molar ratio of 1:2 as the following process: the recombinant

VEGF protein buffer (20 mM Tris, 150 mM NaCl, pH 8.0) was replaced with PBS through ultrafiltration in a 5000 MW ultrafiltration centrifugal tube, and 567.57 μ g of protein was obtained as measured by UV quantification, and the resulting proteins were mixed with 20 mM biotin solution in a 1:2 molar ratio for incubation for 30 min at room temperature in the dark, then filtered again in a 5000 MW ultrafiltration centrifugal tube to remove the unlabeled biotin. After UV quantification, the biotin-labeled proteins were obtained by adding an equal volume of glycerol and a final concentration of 0.1% BSA. The concentration of the VEGF165 protein was 2.08 mg/mL, detected by UV.

Then the affinities of VEGF165-H988 and Avastin in different concentrations with biotinylated recombinant human VEGF proteins were measured, and the obtained KD values were the final affinities.

The results shown in **Table 3** demonstrate that, the binding affinity KD value of VEGF165-H988 to recombinant human VEGF165 protein was 19.5 pM, the binding constant k_{on} value was $3.44E+05 \text{ M}^{-1}\text{s}^{-1}$, and the dissociation constant k_{dis} value was $6.70E-06 \text{ s}^{-1}$; the binding affinity KD value of Avastin to VEGF protein was 29.2E-11 pM, with a binding constant k_{on} value of $1.87E+05 \text{ M}^{-1}\text{s}^{-1}$ and a dissociation constant k_{dis} value of $5.46E-06 \text{ s}^{-1}$, as shown in **Table 3**. From the results, it can be concluded that the affinity of VEGF165-H988 is slightly higher than that of Avastin, i.e. about 1.5 times higher than the affinity of Avastin.

Table 3: The binding affinities of VEGF-H988 and Avastin with recombinant protein VEGF165

Sample	KD(M)	$k_{on}(\text{M}^{-1}\text{s}^{-1})$	$k_{dis}(\text{s}^{-1})$
VEGF-H988	1.95E-11	3.44E+05	6.70E-06
Avastin	2.92E-11	1.87E+05	5.46E-06

3.1.3 Determination of Species Cross Reactivity of humanized

antibody VEGF165-H988

Recombinant human VEGF165 proteins or recombinant mouse mVEGF164 proteins were diluted to 0.1 $\mu\text{g/mL}$, 1 $\mu\text{g/mL}$ and 10 $\mu\text{g/mL}$, respectively, and coated on a 96-well plate overnight at 4°C in 100 μL /well. The plate was washed the next day, blocked at room temperature for 1 h. 100 μL of VEGF165-H988, Avastin (from Roche) or negative control antibody H7N9-R1 was added respectively in a concentration of 1 $\mu\text{g/mL}$ and incubated for 1 h. The plate was washed to remove unbound antibodies. The plate was incubated with goat F(ab')₂ anti-human IgG F(ab')₂/HRP (Jackson ImmunoResearch Laboratories, Inc.) and then repeatedly washed, and the chromogenic substrate solution was added for color development. OD₄₅₀ was measured after the color development was ended. Taking the protein concentration as the horizontal coordinate and the OD₄₅₀ value as the vertical coordinate, the graphPad Prism 6.0 software was used for generating a bar chart.

The results shown in Figure 4 demonstrate that VEGF165-H988 binds to recombinant human VEGF165 protein specifically and show cross-binding with recombinant mouse mVEGF164 protein.

3.2 Receptor blocking properties of humanized antibody VEGF-H988

VEGF165 protein (from SinoBiological, Inc.) at a concentration of 1 $\mu\text{g/mL}$ was coated on a 96-well plate in 100 μL /well overnight at 4°C. The plate was washed the next day and blocked at room temperature for 1 h. 100 μL of 2 $\mu\text{g/mL}$ VEGFR2-his protein (from SinoBiological, Inc.) was added in each well and different concentrations of antibody VEGF-H988, EYLEA, Avastin (from Roche) or negative control antibody H7N9-R1 was added respectively and co-incubated. The plate was washed to remove unbound antibodies. The plate was incubated with C-his-R023/HRP and then repeatedly washed, and the chromogenic

substrate solution was added for color development. OD₄₅₀ was measured after the color development was stabilized, with each group tested in duplicate. Taking the concentration of the antibody as the horizontal coordinate and the inhibition rate PI% as the vertical coordinate, the graphPad Prism 6.0 software was used for data analysis and generating a curve chart to calculate the IC₅₀ value. Inhibition rate (%) = (OD_{blank} - OD_{sample}) / OD_{blank} × 100%, where OD_{blank} indicates the OD value of the wells with only VEGFR2-his added but no humanized antibody added, and OD_{sample} indicates the OD value of the wells with both VEGFR2-his and the humanized antibodies added.

As shown in Figure 5, VEGFR2 protein could effectively bind to the coated VEGF165 protein, and the antibodies VEGF-H988 could effectively inhibit the binding of VEGFR2 protein to VEGFR165 protein, with a relatively weaker inhibitory ability than EYLEA but greater than Avastin, and the negative control antibody has no inhibitory effect.

3.3 Inhibition of the proliferation of HUVEC cells by humanized antibody

3.3.1 Neutralization effect of VEGF165 in different concentrations by humanized antibody VEGF-H988

The effect of VEGF-H988 neutralizing the VEGF 165-induced HUVEC cells proliferation was detected by using the WST-8 method. HUVEC cells were inoculated into a 96-well plate at 4×10³ cells/well, cultured in M199 medium containing 10% FBS and 5% L-Gln for 4 h, and then VEGF-H988, EYLEA or Avastin (from Roche) in different concentrations were added in 50 μL/well, then VEGF-165 at the final concentrations of 1000 ng/mL, 100 ng/mL or 10 ng/mL were added in 10 μL/well, the 96-well plate was incubated in a 37°C, 5% CO₂ cell incubator for 3 days, and the blank well B (no cells), negative control M (cells inoculated, no antibody sample added, VEGF-165 added) and M' (cells inoculated, no antibody sample added and no VEGF-165 added)

were used. After incubation, 10 μ L/well of WST-8 chromogenic solution was added, and the 96-well plate was incubated in CO₂ incubator for color development, OD₄₅₀ and OD₆₃₀ were measured with a microplate reader after the color development was stabilized. For each well, the reading value was (OD₄₅₀ – OD₆₃₀), and the neutralization rate of the antibody was calculated as the OD value for each group was defined as the reading value of the group minus the reading value of blank well B, the neutralization rate% = (OD value of negative control M – OD value of sample) / (OD value of negative control M – OD value of M') $\times$ 100%.

The standard curve was calculated using the automatic analysis function of the statistical software GraphPad Prism, taking the antibody sample concentration as the horizontal coordinate and the neutralization rate as the vertical coordinate, and the 4-parameter logistic regression equation was used to fit the standard “S” curve to calculate the half maximal effective concentration (EC₅₀) of the antibody sample.

As shown in Figures 6 and 7, antibody VEGF-H988 can effectively reduce the ability of VEGF165 to promoting HUVEC proliferation. The neutralizing ability of VEGF-H988 was stronger than that of EYLEA and Avastin at different concentrations of recombinant human VEGF165; and the difference of the neutralizing ability was stronger as the concentrations of VEGF165 is higher. Under the condition of high concentrations of VEGF165, VEGF-H988 still has a low neutralizing EC₅₀ and could still maintain the maximum neutralization rate, while the neutralizing EC₅₀ of EYLEA and Avastin gradually increased and was accompanied by a decrease of the maximum neutralization rate. The half maximal effective concentrations (EC₅₀) and maximum neutralization rates of each antibody neutralizing VEGF165 in different concentrations are summarized in Table 4.

Table 4: EC₅₀ and maximum neutralization rate of VEGF-H988 neutralizing VEGF-165 of different concentrations

VEGF165 concentration	Sample	EC ₅₀ (nM)	maximum neutralization rate (%)
1000ng/mL	VEGF-H988	9.67	103.7
	EYLEA	40.18	80.2
100ng/mL	VEGF-H988	1.34	102.3
	EYLEA	2.90	98.7
10ng/mL	VEGF-H988	0.14	100.2
	EYLEA	0.16	100.7
1000ng/mL	VEGF-H988	9.33	104.5
	Avastin	42.49	88.1
100ng/mL	VEGF-H988	1.36	102.1
	Avastin	4.40	91.3
10ng/mL	VEGF-H988	0.13	100.5
	Avastin	0.24	92.5

3.3.2 Neutralization effect of different subtypes of VEGFs by humanized antibody VEGF-H988

5 The WST-8 method was used to detect the effect of VEGF-H988 neutralizing different subtypes of VEGF (VEGF165, VEGFC and VEGFD) -induced HUVEC cells proliferation. HUVEC cells were inoculated into a 96-well plate at 4×10^3 cells/well, cultured in M199 medium containing 10% FBS and 5% L-Gln for 4 h, and then different

10 concentrations of VEGF-H988 or Avastin (from Roche) were added in 50 μ L/well, then VEGF-165, VEGFC and VEGFD mixture (the final concentrations were 25 ng/mL、1000 ng/mL、6000 ng/mL respectively) was added in 10 μ L/well, the 96-well plate was incubated in a 37°C, 5% CO₂ cell incubator for 3 days, and the blank well B (no cells), negative

15 control M (cells inoculated, no antibody sample added, VEGFs added) and M'(cells inoculated, no antibody sample and no VEGFs) were used. After incubation, 10 μ L/well of WST-8 chromogenic solution was added, and the 96-well plate was incubated in CO₂ incubator for color development,

OD₄₅₀ and OD₆₃₀ were measured with a microplate reader after the color development was stabilized. For each well, the reading value was (OD₄₅₀ – OD₆₃₀), and the neutralization rate of the antibody was calculated as the OD value for each group was defined as the reading value of the group minus the reading value of blank well B, the neutralization rate% = (OD value of negative control M – OD value of sample) / (OD value of negative control M – OD value of M') × 100%. The standard curve was calculated using the automatic analysis function of the statistical software GraphPad Prism, taking the antibody sample concentration as the horizontal coordinate and the neutralization rate as the vertical coordinate, and the 4-parameter logistic regression equation was used to fit the standard “S” curve to calculate the half maximal effective concentration (EC₅₀) of the antibody sample.

As shown in Figure 8, in the case of different subtypes of VEGF (VEGF165, VEGFC, VEGFD) acting simultaneously on HUVEC cells, antibody VEGF-H988 has a stronger neutralizing effect than Avastin. In the concentration range of 0.016-4.000 nM, VEGF-H988 has a smaller EC₅₀ than Avastin, being 0.26 nM and 0.77 nM, respectively; and VEGF-H988 has a higher neutralization rate than Avastin, being 86.1% and 58.5% respectively. The half maximal effective concentrations (EC₅₀) and the maximum neutralization rates of VEGF and Avastin to neutralize different subtypes of VEGF are summarized in Table 5.

Table 5. EC₅₀ and maximum neutralization rate of VEGF-H988 neutralizing different subtypes of VEGF

Antibody	EC ₅₀ (nM)	Maximum neutralization rate
		E _{max} (%)
VEGF-H988	0.26	86.1
Avastin	0.77	58.5

Example 4: Repeated administration toxicity study of antibody VEGF-H988 in mice

CD-1 mice, 8 each in the aged group (35 weeks of age) and normal group (9 weeks of age), half male and half female, were used. The mice were divided into four groups (G1-G4) according to the complete randomization method, with four mice in each group and half of the males and half of the females. The specific grouping was as follows: G1: old mice solvent control group; G2: old mice study group; G3: normal mice solvent control group; G4: normal mice study group. The VEGF-H988 antibodies were repeatedly administered to the study groups, and the control groups were given an equal volume of solvent. The dose was 50 mg/kg, the volume of each administration was 5 mL/kg (1st to 8th dose) and 10 mL/kg (9th to 16th dose), respectively; the mode of administration was intraperitoneal injection, with a frequency of twice weekly. All mice were subjected to orbital blood sampling before and 0.5 h, 2 h, 4 h, 6 h, 24 h, 48 h, 72 h after the first dosing as well as before and 1 h after every other dosing, for pharmacokinetic and immunogenicity assays. The body weights of mice were measured and recorded before each dosing. The detailed dosing regimen is shown in Table 6.

Table 6. Dosing regimen of antibody VEGF-H988

Group	Antibody	Dose	Mode of administration	Frequency of administration	Mice status
G1	None (solvent control group)	0 mg/kg	i.p.	Twice weekly	2♀ & 2♂ (aged)
G2	VEGF-H988	50 mg/kg	i.p.	Twice weekly	2♀ & 2♂ (aged)
G3	None (solvent control group)	0 mg/kg	i.p.	Twice weekly	2♀ & 2♂

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					(normal age)
G4	VEGF-H988	50 mg/kg	i.p.	Twice weekly	2♀ & 2♂ (normal age)

The body weights of the mice in the antibody VEGF-H988 groups were similar to those of the corresponding solvent control groups, indicating that the antibody VEGF-H988 had no effect on the body weight of the mice. During the study, the clinical abnormalities were observed in the antibody VEGF-H988 groups. All mice were subjected to gross autopsy, and there were no visible abnormalities in the tissues and organs. Histopathological examination showed that all mice had venous congestion in the liver, lungs, and kidneys, but there was no significant difference between the control groups and the study groups. Sparse individual mice showed pink serous or erythrocyte stasis in the spleen and the inflammatory cell infiltration in the kidneys, which were considered to be individual difference and not related to drug administration. The toxicokinetic parameters of mice after the first dose are summarized in **Table 7**.

Table 7. Toxicokinetic parameters of toxicity study of repeated administration in mice

Group	Gen-der	/	t _{1/2} (h)	T _{max} (h)	C _{max} (μg/mL)	AUC _{last} (h×μg/mL)	AUC _{0-∞} (h×μg/mL)	V _{z_F} (mL/kg)	Cl _F (mL/h/kg)	MRT _{last} (h)
G2 (Aged)	♂	Mean	53.246	4.000	408.899	11735.125	14696.594	263.934	3.469	26.128
		SD	5.161	0.000	63.837	1381.429	2880.380	26.398	0.680	4.929
	♀	Mean	55.751	2.000	435.691	12033.935	15499.970	254.922	3.276	26.934
		SD	20.717	0.000	25.122	975.896	2717.582	51.721	0.574	0.748

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	♀&♂	Mean	54.499	3.000	422.295	11884.530	15098.282	259.428	3.372	26.531
		SD	12.411	1.155	42.521	991.632	2332.899	33.927	0.526	2.916
G4 (Normal age)	♂	Mean	50.699	4.000	299.521	11327.725	14664.044	254.388	3.475	31.698
		SD	0.516	0.000	47.342	2175.405	2832.204	51.669	0.671	0.440
	♀	Mean	80.867	4.000	202.054	9150.110	15211.402	469.071	3.873	37.243
		SD	11.207	0.000	143.203	5988.595	8367.579	311.174	2.130	1.557
	♀&♂	Mean	65.783	4.000	250.788	10238.918	14937.723	361.730	3.674	34.471
		SD	18.583	0.000	103.679	3887.487	5110.035	220.294	1.310	3.335

As shown in the blood drug concentration-time curve in Figures 9-11, there was no significant difference in the trend of blood concentration changes between male and female animals in each group. There was no significant accumulation after multiple dosing in both groups, which may be related to the higher dose administered groups having reached saturation levels. The peak blood concentrations in the G2 group (aged) and the G4 group (normal) were basically the same, and the trough concentrations in the aged group were slightly lower, which may be related to the large individual differences within the group.

Mice in each group showed no significant increase or even some decrease in the blood drug concentration at 1 h after administration, which did not correlate well with immunogenicity (see **Table 8** for immunogenicity test data). In addition, the abnormal blood drug concentration at 1 h after administration may also be related to the drug distribution speed *in vivo* and individual differences.

Although the immunogenicity test results showed positives in part of subjects, the difference between the SNR and the threshold SCP was small, actually, they are very closely, thus a strong positive is not shown, and it is considered that the aforesaid positive results were probably false positives. **Table 8** summarizes the immunogenicity results of the repeated dosing toxicity study of the antibody VEGF-H988 in mice.

Table 8. Immunogenicity test results of toxicity study of repeated

administration of VEGF-H988 in mice

Time point		0 h		432 h	
Group	Mouse No.	SNR	Conclusion	SN R	Conclusion
G2 (Aged)	69	0.791	-	0.814	-
	78	1.140	+	0.919	-
	64	0.791	-	0.791	-
	63	1.058	+	0.977	-
G4 (Normal)	88	0.814	-	0.860	-
	87	0.767	-	0.895	-
	89	0.884	-	1.070	+
	84	0.791	-	0.907	-

Repeated administration toxicity tests in mice showed that no significant drug-related toxic effects were observed when the antibody VEGF-H988 was repeatedly administered at a dose of 50 mg/kg twice weekly via intraperitoneal injection to aged CD-1 mice and normal CD-1 mice for 16 doses.

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Example 5: Efficacy study in human colorectal cancer cell line HCT-116 xenograft tumor model

Female Balb/c-nu nude mice were from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Animal production license No. SCXK (Beijing) 2016-0006, Quality certificate No. 11400700373019). HCT116 tumor blocks were obtained from mice inoculated with self-developed HCT116 cell line. Balb/c-nu 6-week-old female mice were inoculated subcutaneously with HCT116 tumor blocks of approximately 2×2×2 (mm³) in size, and when the tumor volume reached approximately 300 mm³, the mice were administered in groups with intraperitoneal injection of 1 mg/kg of VEGF-H988 or Avastin (from

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SinoCelltech Co., Ltd.) or the corresponding solvent, respectively twice weekly. Tumor volumes were measured twice weekly to assess the antitumor efficacy of VEGF-H988 versus Avastin.

As shown in Figure 12, in the case of a larger initial tumor volume of about 300 mm³ and a lower dose (1mg/kg), the tumor growth rate was progressively reduced in the VEGF-H988-treated group compared to the non-dosed control group since Day 7 after administration. The inhibitory effect of Avastin on tumor growth began on the 11th day after administration, later than that in the equivalent dose of VEGF-H988 treated group. Tumor inhibition assays in the HCT-116 xenograft tumor model showed that the inhibitory effect of VEGF-H988 on tumor growth is better than that of Avastin.

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List of sequences

SEQ ID NO	Identity	Sequence
SEQ ID NO.1	Amino acid sequence of Met 1- Arg191 of the human VEGF protein (UniProtKB P15692)	MNFLLSVHWSLALLLYLHHAKWS QAAPMAEGGGQNHHEVVKFMDVYQRS YCHPIETLVDIFQEYPDEIEYIFKPSCVPL MRCGGCCNDEGLECVPTESNITMQIM RIKPHQGGQHIGEMSFLQHNKCECRPKK DRARQEKKSVRGKGKGQKRKRKKSRY KSWSVYVGARCCCLMPWSLPGPHPCGPC SERR
SEQ ID NO.2	Nucleotide sequence of the linker used in the construction of the phage antibody library for the linkage of the rabbit antibody scFv	TCTAGTGGTGGCGGTGGTTCGGGC GGTGGTGGAGGTGGTAGTTCTAGATC TTCC (SSGGGGSGGGGGGSSRSS)
SEQ ID NO.3	Nucleotide sequence of rabbit antibody scFv which is used in the construction of antibody VEGF165-R859	Nucleotide sequence of light chain variable region of VEGF165-R859 GATGTCGTGATGACCCAGACTGCA GCCTCCGTGTCTGAACCTGTGGGAGG CACAGTCACCATCAAGTGCCAGGCCA GTCAGAGCATTAGGAGTTGGTTATCCT GGTATCAGCAGAAACCAGGGCAGCCT CCCAAGCTCCTGATCTATCAGGCATCC AAATTGGCATCTGGGGTCCCATCGCG GTTCAAAGGCAGTGGATATGGGACAG AGTTCACTCTCACCATCAGCGACCTGG AGTGTGCCGATGCTGCCACTTACTACT GTCAAAACAATTATTCTTTTAGTAAAG

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		ATGGTGGTGCTTTCGGCGGAGGGACC GAGGTGGTCGTCAA
		Linker (SEQ ID NO:2):
		TCTAGTGGTGGCGGTGGTTCGGGC GGTGGTGGAGGTGGTAGTTCTAGATC TTCC
		Nucleotide sequence of heavy chain variable region of VEGF165-R859
		CAGTCGGTGGAGGAGTCCGGGGGT CGCCTGGTCACGCCTGGGACACCCCT GACACTCACCTGCCAAGTCTCTGGATT CTCCCTCAACATCTACGACATGACCTG GGTCCGCCAGGCTCCAGGGAAGGGGC TGGAATGGATCGGAATCATTGCGCCT GATGATAGCGCATACTACGCGAACTG GGCGAAAGGCCGATTCACCATCTCCA AAACCTCGACCACGGTGGATCTGAAA ATGACCAGTCCGACAACCGAGGACAC GGCCACCTATTTCTGTGCCAGAAATGC CTATAGTAGTGGCTGGGGTGGGGACT TGTGGGGCCCAGGCACCCTGGTCACT GTCTCTTCA
SEQ ID NO.4	Nucleotide sequence of the rabbit antibody scFv which is used in the construction of antibody VEGF165-R988	Nucleotide sequence of light chain variable region of VEGF165-R988(SEQ ID NO:8)
		GAGCTCGATCTGACCCAGACTCCA TCCCCCGTGTCTGCGGCTGTTGGAGGC ACAGTCACCATCAATTGCCAGTCCAGT CAGACTATTTATGCTAACAGGCGCTTA GCCTGGTATCAACAGAAACCAGGGCA GCCTCCCAAGCTCCTGATCTATGGTGC

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		ATCCACTCTGGCATCTGGGGTCCCATC GCGGTTCAAAGGCAGTGGATCTGGGA CACAGTTCACTCTCACCATCAGCGGCG TGCAGTGTGACGATGCTGCCACTTACT ACTGTGCAGGCTATAAAAAGTTATGAT GGTGATGATGTTGGTTTCGGCGGAGG GACCGAGGTGGTCGTCAAA
		Linker(SEQ ID NO:2):
		TCTAGTGGTGGCGGTGGTTCGGGC GGTGGTGGAGGTGGTAGTTCTAGATC TTCC
		Nucleotide sequence of heavy chain variable region of VEGF165-R988(SEQ ID NO:7)
SEQ ID NO.5	Nucleotide sequence of the rabbit antibody scFv which is used in the construction	CAGTCGGTGGAGGAGTCCGGGGGT CGCCTGGTAACGCCTGGGACACCCCT GACACTCACCTGCACAGTCTCTGGAAT CGACCTCAGTAGCTATGCAATAAGCT GGGTCCGCCAGGCTCCAGGGAAGGGG CTGGAATACATCGGATACATTTGGAA TGCTGGTAACACCTACTACGCGAGCT GGGCAAAAGGCCGATTCACCATCTCC AAAACCTCGACCACGGTGGATCTGAA AATCACCAGTCCGACAACCGAGGACA CGGCCACCTATTTCTGTGCCAGAGGA ACATTAGGGGACTACAATGGCATGGA CCCCTGGGGCCCAGGGACCCTCGTCA CCGTCTCTTCA
		Nucleotide sequence of light chain variable region of VEGF165-R613
		GAGCTCGTGATGACCCAGACTCCA TCCCCCGTGTCTGCGGCTGTTGGAGGC ACAGTCACCATCAATTGCCAGTCCAGT CAGAGTGTTTATAGTAACAACCGGTT

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	of antibody VEGF165-R613	AGCCTGGTATCAGCAGAAACCAGGGC AGCCTCCCAAGCTCCTGATCTATGGTG CATCCACTCTGGCATCTGGGGTCCCAT CGCGGTTCAAAGGCAGTGGATCTGGG ACACAGTTCACTCTCACCATCAGCGAC GTGCAGTGTGACGATGCTGCCACTTAC TACTGTGCAGGCTATAAAAGTTATGAT GGTGATGATGTTGGTTTCGGCGGAGG GACCGAGGTGGTGGTCAAA
		Linker(SEQ ID NO:2):
		TCTAGTGGTGGCGGTGGTTCGGGC GGTGGTGGAGGTGGTAGTTCTAGATC TTCC
		Nucleotide sequence of heavy chain variable region of VEGF165-R613
SEQ ID NO.6	Nucleotide sequence of the rabbit antibody scFv which is used in the construction	CAGTCGGTGGAGGAGTCCGGGGGT CGCCTGGTCACGCCTGGGACACCCCT GACACTCACCTGCACAGTCTCTGGAAT CGACCTCAGTAGCTATGCAATAAGCT GGGTCCGCCAGGCTCCAGGGAAGGGG CTGGAATACATCGGATACATTTGGAG TACTGATAACACCTACTATGCGAGCTG GGCAAAGGCCGATTCACCATCTCCG AGGCCTCGACCACGGTGGATCTGAAA ATCACCAGCCCGACAACCGAGGACAC GGCCACCTATTTCTGTGCCAGAGGAA CGTTAGGGGACTACAATGGCATGGAC CCCTGGGGCCCAGGACCCTCGTCAC CGTCTCTTCA
		Nucleotide sequence of light chain variable region of VEGF165-R812
		GAGCTCGTGCTGACCCAGACTCCA GCCTCCGTGGAGGCAGCTGTGGGAGG CACAGTCACCATCAAGTGCCAGGCCA

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	of antibody VEGF165-R812	GTCAGAGCATTAATAGTTGGTTATCCT GGTATCAGCAGAAACCAGGGCAGCGT CCCAAGCTCCTGATCTACCAGGCATCC AAACTGGCATCTGGGGTCCCATCGCG GTTCAAAGGCAGTGGATCTGGGACAG AGTACACTCTCACCATCAGCGACCTG GAGTGTGCCGATGCTGCCACTTATTAC TGCCAGAACAATCTTGGTGGTGGTGA TGGTAGTTATGGTCTTCCTTTCGGCGG AGGGACCGAGGTGGTGGTCAAA
		Linker(SEQ ID NO:2):
		TCTAGTGGTGGCGGTGGTTCGGGC GGTGGTGGAGGTGGTAGTTCTAGATC TTCC
		Nucleotide sequence of heavy chain variable region of VEGF165-R812
		CAGTCGTTGGAGGAGTCCGGGGGT CGCCTGGTAACGCCTGGAGGCTCCCT GACACTCACCTGCACAGCCTCTGGATT CGACCTCGGTATCTATGAAATAACCTG GGTCCGCCAGGCTCCAGGGAAGGGGC TGGAATGGATCGGAGTCATTTATGGT GATGGTGACACAGTCTACGCGAACTG GGCGAAAGGCCGATTCACCATCTCCA AAACCTCGACCACGGTGGATCTGAAA ATCTCCAGTCCGACAACCGAGGACAC GGCCACCTATTTCTGTGCCAGAAATGG CTATACTACTGGCTGGGGTGGGGACTT GTGGGGCCCAGGCACCCTGGTCACTG TCTCTTCA
SEQ ID NO:7	Nucleotide sequence of heavy chain variable region of the	CAGTCGGTGGAGGAGTCCGGGGGT CGCCTGGTAACGCCTGGGACACCCCT GACACTCACCTGCACAGTCTCTGGAAT CGACCTCAGTAGCTATGCAATAAGCT

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	rabbit antibody VEGF165-R988	GGGTCCGCCAGGCTCCAGGGAAGGGG CTGGAATACATCGGATACATTTGGAA TGCTGGTAACACCTACTACGCGAGCT GGGCAAAGGCCGATTCACCATCTCC AAAACCTCGACCACGGTGGATCTGAA AATCACCAGTCCGACAACCGAGGACA CGGCCACCTATTTCTGTGCCAGAGGA ACATTAGGGGACTACAATGGCATGGA CCCCTGGGGCCCAGGGACCCTCGTCA CCGTCTCTTCA
SEQ ID NO:8	Nucleotide sequence of light chain variable region of the rabbit antibody VEGF165-R988	GAGCTCGATCTGACCCAGACTCCA TCCCCCGTGTCTGCGGCTGTTGGAGGC ACAGTCACCATCAATTGCCAGTCCAGT CAGACTATTTATGCTAACAGGCGCTTA GCCTGGTATCAACAGAAACCAGGGCA GCCTCCCAAGCTCCTGATCTATGGTGC ATCCACTCTGGCATCTGGGGTCCCATC GCGGTTCAAAGGCAGTGGATCTGGGA CACAGTTCACTCTCACCATCAGCGGCG TGCAGTGTGACGATGCTGCCACTTACT ACTGTGCAGGCTATAAAAGTTATGAT GGTGATGATGTTGGTTTCGGCGGAGG GACCGAGGTGGTCGTCAA
SEQ ID NO:9	Nucleotide sequence of the heavy chain constant region of rabbit IgG1	GGTCAACCTAAGGCTCCGTCAGTC TTCCCACTGGCCCCCTGCTGCGGGGAC ACACCCAGCTCCACGGTGACCCTGGG CTGCCTGGTCAAAGGCTACCTCCCGG AGCCAGTGACCGTGACCTGGAACCTCG GGCACCCCTACCAATGGGGTACGCAC CTTCCCGTCCGTCCGGCAGTCCTCAGG CCTCTACTCGCTGAGCAGCGTGGTGA GCGTGACCTCAAGCAGCCAGCCCGTC ACCTGCAACGTGGCCCAACCAGCCAC CAACACCAAAGTGGACAAGACCGTTG

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		CGCCCTCGACATGCAGCAAGCCCACG TGCCCACCCCCTGAACTCCTGGGGGG ACCGTCTGTCTTCATCTTCCCCCAA ACCCAAGGACACCCTCATGATCTCAC GCACCCCCGAGGTACATGCGTGGTG GTGGACGTGAGCCAGGATGACCCGA GGTGCAGTTCACATGGTACATAACA ACGAGCAGGTGCGCACCGCCCGGCCG CCGCTACGGGAGCAGCAGTTCAACAG CACGATCCGCGTGGTCAGCACCTCCC CATCGCGCACCAAGGACTGGCTGAGGG GCAAGGAGTTCAAGTGCAAAGTCCAC ACAAGGCACTCCCGGCCCCCATCGA GAAAACCATCTCCAAAGCCAGAGGGC AGCCCCTGGAGCCGAAGGTCTACACC ATGGGCCCTCCCCGGGAGGAGCTGAG CAGCAGGTCGGTCAGCCTGACCTGCA TGATCAACGGCTTCTACCCTTCCGACA TCTCGGTGGAGTGGGAGAAGAACGGG AAGGCAGAGGACAACCTACAAGACCAC GCCGGCCGTGCTGGACAGCGACGGCT CCTACTTCTCTACAGCAAGCTCTCAG TGCCCACGAGTGAGTGGCAGCGGGGC GACGTCTTCACCTGCTCCGTGATGCAC GAGGCCTTGCAACAACCACTACACGCA GAAGTCCATCTCCCGCTCTCCGGGTAA ATAA
SEQ ID NO:10	Nucleotide sequence of the constant region of the rabbit kappa light chain	GGGGATCCAGTTGCACCTACTGTC CTCATCTTCCCACCAGCTGCTGATCAG GTGGCAACTGGAACAGTCACCATCGT GTGTGTGGCGAATAAATACTTTCCCGA TGTCACCGTCACCTGGGAGGTGGATG GCACCACCCAAACAACCTGGCATCGAG AACAGTAAAACACCGCAGAATTCTGC

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		AGATTGTACCTACAACCTCAGCAGCA CTCTGACACTGACCAGCACACAGTAC AACAGCCACAAAGAGTACACCTGCAA GGTGACCCAGGGCACGACCTCAGTCG TCCAGAGCTTCAATAGGGGTGACTGTT AA
SEQ ID NO:11	Amino acid sequence of the heavy chain variable region of rabbit antibody VEGF165-R988	QSVEESGGRLVTPGTPLTLTCTVSGI DLSSYAISWVRQAPGKGLEYIGYIWNA GNTYYASWAKGRFTISKTSTTVDLKITS PTTEDTATYFCARGTLGDYNGMDPWGP GTLVTVSS
SEQ ID NO:12	Amino acid sequence of the light chain variable region of rabbit antibody VEGF165-R988	ELDLTQTPSPVSAAVGGTVTINCQSS QTIYANRRRLAWYQQKPGQPPKLLIYGA STLASGVPSRFRKSGSGTQFTLTISGVQC DDAATYYCAGYKSYDGDDVGFGGGTE VVVK
SEQ ID NO:13	Amino acid sequence of light chain CDR1 of the rabbit antibody VEGF165-R988	QSSQTIYANRRLA
SEQ ID NO:14	Amino acid sequence of light chain CDR2 of the rabbit antibody VEGF165-R988	GASTLAS
SEQ ID NO:15	Amino acid sequence of light chain CDR3 of the rabbit antibody VEGF165-R988	AGYKSYDGDDVG

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SEQ ID NO:16	Amino acid sequence of heavy chain CDR1 of the rabbit antibody VEGF165-R988	GIDLSSY AIS
SEQ ID NO:17	Amino acid sequence of heavy chain CDR2 of the rabbit antibody VEGF165-R988	YIWNAGNTYYASWAKG
SEQ ID NO:18	Amino acid sequence of heavy chain CDR3 of the rabbit antibody VEGF165-R988	ARGTLGDYNGMDP
SEQ ID NO:19	Nucleotide sequence of the humanized antibody VEGF165-H988- 10 heavy chain containing the signal peptide	Nucleotide sequence of heavy chain signal peptide(SEQ ID NO:45):
		ATGGGCTGGTCCCTGATTCTGCTGT TCCTGGTGGCTGTGGCTACCAGGGTGC TGAGT
		Nucleotide sequence of heavy chain variable region(SEQ ID NO:23):
		CAGTCTGTCCAGGAGTCTGGACCTGGACTGGTGAAG CCATCTGAGACCCTGTCCCTGACTTGACTGTGTCTGGC ATTGACCTGTCCTCCTATGCCATCTCCTGGGTGAGACAA CCTCCTGGCAAGGGATTGGAATACATTGGCTACATCTGG AATGCTGGCAACACCTACTATGCCTCCTGGGCTAAGGGC AGGGTGACCATCTCTGTGGACACCAGCAAGAACCAGGTG GACCTGAAACTGTCCTCTGTGACAGCAGCAGACACAGCA GTCTACTTCTGTGCCAGGGGCACCCTGGGAGACTACAAT GGGATGGACCCATGGGGACCTGGCACCCCTGGTGACAGTG TCCAGC

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		Nucleotide sequence of the human IgG1 heavy chain constant region (SEQ ID NO:49): GCTAGCACCAAGGGCCCATCGGTC TCCCCCTGGCACCCCTCCTCCAAGAGC ACCTCTGGGGGACAGCGGCCCTGGG CTGCCTGGTCAAGGACTACTTCCCCGA ACCGGTGACGGTGTCGTGGAATCAG GCGCCCTGACCAGCGGCGTGCACACC TTCCCGGCTGTCCTACAGTCCTCAGGA CTCTACTCCCTCAGCAGCGTGGTGACC GTGCCCTCCAGCAGCTTGGGCACCCA GACCTACATCTGCAACGTGAATCACA AGCCCAGCAACACCAAGGTGGACAAG AAAGTTGAGCCCAAATCTTGTGACAA AACTCACACATGCCCACCGTGCCCAG CACCTGAACTCCTGGGGGGACCGTCA GTCTTCCTCTTCCCCC AAAACCCAAG GACACCCTCATGATCTCCCGGACCCCT GAGGTCAC_gTGCGTGGTGGTGGACGT GAGCCACGAAGACCC_cGAGGTCAAGT TCAACTGGTACGTGGACGGCGTGGAG GTGCATAATGCCAAGACAAAGCCGCG GGAGGAGCAGTACAACAGCACGTACC GTGTGGTCAGCGTCCTCACCGTCCTGC ACCAGGACTGGCTGAATGGCAAGGAG TACAAGTGCAAGGTCTCCAACAAAGC CCTCCCAGCCCCCATCGAGAAAACCA TCTCCAAAGCCAAAGGGCAGCCCCGA GAACCACAGGTGTACACCCTGCCCCC ATCCCGGGATGAGCTGACCAAGAACC AGGTCAGCCTGACCTGCCTGGTCAAA GGCTTCTATCCCAGCGACATCGCCGTG GAGTGGGAGAGCAATGGGCAGCCGGA
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		GAACAAC TACAAGACCACGCCTCCCG TGCTGGACTCCGACGGCTCCTTCTTCC TCTACAGCAAGCTCACCGTGGACAAG AGCAGGTGGCAGCAGGGGAACGTCTT CTCATGCTCCGTGATGCATGAGGCTCT GCACAACCACTACACGCAGAAGAGCC TCTCCCTGTCTCCGGGTAAATGA
SEQ ID NO:20	Nucleotide sequence of humanized antibody VEGF165-H988- 10 light chain containing signal peptide	Nucleotide sequence of the light chain signal peptide(SEQ ID NO:46):
		ATGGGCTGGTCCTGTATCATCCTGT TCCTGGTGGCTACAGCCACAGGAGTG CATAGT
		Nucleotide sequence of the light chain variable region(SEQ ID NO:24):
		GAACTCCA ACTTACCCAGAGCCCA TCCTCCCTGTCTGCCTCTGTGGGAGAC AGGGTGACCATCACTTGTCAGTCCAG CCAGACCATCTATGCCAACAGGAGAC TGGCTTGGTATCAACAGAAGCCTGGC AAGGTGCCAAA ACTGCTGATTTATGG AGCCAGCACCCCTGGCATCTGGAGTGC CAAGCAGGTTCAAGGGCTCTGGCTCT GGCACAGACTTCACCCTGACCATCTCC TCCCTCCAACCTGAGGATGTGGCTACC TACTACTGTGCTGGCTACAAGTCCTAT GATGGAGATGATGTGGGCTTTGGAGG AGGCACCAAGGTGGAGATTAAG
		Nucleotide sequence of the human kappa light chain constant region(SEQ ID NO:50):
		CGTACGGTGGCTGCACCATCTGTCT TCATCTTCCCGCCATCTGATGAGCAGT TGAAATCTGGA ACTGCCTCTGTTGTGT GCCTGCTGAATAACTTCTATCCCAGAG AGGCCAAAGTACAGTGGAAGGTGGAT

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		AACGCCCTCCAATCGGGTAACTCCCA GGAGAGTGTACAGAGCAGGACAGCA AGGACAGCACCTACAGCCTCAGCAGC ACCCTGACGCTGAGCAAAGCAGACTA CGAGAAACACAAAGTCTACGCCTGCG AAGTCACCCATCAGGGCCTGAGCTCG CCCGTCACAAAGAGCTTCAACAGGGG AGAGTGTTAG
SEQ ID NO:21	Amino acid sequence of the heavy chain of the humanized antibody VEGF165-H988- 10 containing the signal peptide	Amino acid sequence of the heavy chain signal peptide(SEQ ID NO:37):
		MGWSLILLFLVAVATRVLS
		Amino acid sequence of the heavy chain variable region(SEQ ID NO:25):
		QSVQESGPGLVKPSETLSLTCTVSGI DLSSYAISWVRQPPGKGLEYIGYIWNAG NTYYASWAKGRVTISVDTSKNQVDLKL SSVTAADTAVYFCARGTLGDYNGMDP WGPGLTVTVSS
		Amino acid sequence of the human IgG1 heavy chain constant region(SEQ ID NO:41):
		ASTKGPSVFPLAPSSKSTSGGTAALG CLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVVTVPSSSLGTQTYI CNVNHKPSNTKVDKKVEPKSCDKTHTC PPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDG VEVHNAKTKPREEQYNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKT ISKAKGQPREPQVYTLPPSRDELTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNY KTTTPVLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSP GK

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SEQ ID NO:22	Amino acid sequence of the light chain of the humanized antibody VEGF165-H988- 10 containing the signal peptide	Amino acid sequence of the light chain signal peptide(SEQ ID NO:38):
		MGWSCILFLVATATGVHS
		Amino acid sequence of the light chain variable region(SEQ ID NO:26):
		ELQLTQSPSSLSASVGDRVITTCQSS QTIYANRRRLAWYQQKPGKVPKLLIYGA STLASGVPSRFKSGSGTDFTLTISSLQP EDVATYYCAGYKSYDGDVDVGFGGGK VEIK
		Amino acid sequence of the human kappa light chain constant region(SEQ ID NO:42):
		RTVAAPSVFIFPPSDEQLKSGTASVV CLLNNFYPREAKVQWKVDNALQSGNS QESVTEQDSKDYSLSTLTLSKADYE KHKVYACEVTHQGLSSPVTKSFNRGEC
SEQ ID NO:23	Nucleotide sequence of the heavy chain variable region of humanized antibody VEGF165-H988- 10	CAGTCTGTCCAGGAGTCTGGACCTGGACTGGTGAAG CCATCTGAGACCCTGTCCCTGACTGTACTGTGTCTGGC ATTGACCTGTCTCCTATGCCATCTCCTGGGTGAGACAA CCTCCTGGCAAGGGATTGGAATACATTGGCTACATCTGG AATGCTGGCAACACCTACTATGCCTCCTGGGCTAAGGGC AGGGTGACCATCTCTGTGGACACCAGCAAGAACCAGGTG GACCTGAACTGTCCTCTGTGACAGCAGCAGACACAGCA GTCTACTTCTGTGCCAGGGGCACCCTGGGAGACTACAAT GGGATGGACCCATGGGGACCTGGCACCCCTGGTGACAGTG TCCAGC
SEQ ID NO:24	Nucleotide sequence of the light chain variable region of the humanized antibody	GAACTCCAACCTTACCCAGAGCCCA TCCTCCCTGTCTGCCTCTGTGGGAGAC AGGGTGACCATCACTTGTCAGTCCAG CCAGACCATCTATGCCAACAGGAGAC TGGCTTGGTATCAACAGAAGCCTGGC AAGGTGCCAAAACCTGCTGATTTATGG AGCCAGCACCCCTGGCATCTGGAGTGC

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	VEGF165-H988-10	CAAGCAGGTTCAAGGGCTCTGGCTCTGGCACAGACTTCACCCTGACCATCTCCTCCCTCCAACCTGAGGATGTGGCTACCTACTACTGTGCTGGCTACAAGTCCTATGATGGAGATGATGTGGGCTTTGGAGGAGGCACCAAGGTGGAGATTAAG
SEQ ID NO:25	Amino acid sequence of the heavy chain variable region of the antibody VEGF165-H988-10	QSVQESGPGLVKPSETLSLTCTVSGIDLSSYAISWVRQPPGKGLEYIGYIWNAGNTYYASWAKGRVTISVDTSKNQVDLKLSSVTAADTAVYFCARGTLGDYNGMDPWGPGLVTVSS
SEQ ID NO:26	Amino acid sequence of the light chain variable region of the antibody VEGF165-H988-10	ELQLTQSPSSLSASVGDRVITTCQSSQTIYANRRRLAWYQQKPGKVPKLLIYGASTLASGVPSRFRKSGSGTDFTLTISSLQPEDVATYYCAGYKSYDGDDVGFGGGTVK
SEQ ID NO:27	Amino acid sequence of light chain CDR1 of humanized antibody VEGF-H988	QSSKFLWQGRRLA
SEQ ID NO:28	Amino acid sequence of light chain CDR2 of humanized antibody VEGF-H988	GASTLAS
SEQ ID NO:29	Amino acid sequence of light	AGYKSYDGDVVG

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	chain CDR3 of humanized antibodyVEGF- H988	
SEQ ID NO:30	Amino acid sequence of heavy chain CDR1 of humanized antibodyVEGF- H988	GIDLSSYAIS
SEQ ID NO:31	Amino acid sequence of heavy chain CDR2 of humanized antibodyVEGF- H988	YIWNDLFTYYASWAKG
SEQ ID NO:32	Amino acid sequence of heavy chain CDR3 of humanized antibodyVEGF- H988	ARGTLGDYGGMDP
SEQ ID NO:33	Amino acid sequence of the	Amino acid sequence of the heavy chain variable region (SEQ ID NO:39):

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	heavy chain of humanized antibody VEGF-H988	QSVQESGPGLVKPSETLSLTCTVSGI DLSSYAISWVRQPPGKGLEYIGYIWN DL FTYYASWAKGRVTISVDTSKNQVDLKL SSVTAADTAVYFCARGTLGDYGGMDP WGQGTLVTVSS
		Amino acid sequence of the human IgG1 heavy chain constant region (SEQ ID NO:41):
		ASTKGPSVFPLAPSSKSTSGGTAALG CLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVVTVPSSSLGTQTYI CNVNHKPSNTKVDKKVEPKSCDKTHTC PPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDG VEVHNAKTKPREEQYNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKT ISKAKGQPREPQVYTLPPSRDELTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSP GK
SEQ ID NO:34	Amino acid sequence of the light chain of humanized antibody VEGF-H988	Amino acid sequence of the light chain variable region(SEQ ID NO:40):
		ELQLTQSPSSLSASVGDRVTITCQSS KFLWQGRRLAWYQQKPGKVPKLLIYG ASTLASGVPSRFRKGSGSGTDFLTISLQ PEDVATYYCAGYKSYDGDVVGFGGGT KVEIK
		Amino acid sequence of the human kappa light chain constant region(SEQ ID NO:42):
		RTVAAPSVFIFPPSDEQLKSGTASVV CLLNNFYPREAKVQWKVDNALQSGNS

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		QESVTEQDSKDYSLSSTLTLSKADYE KHKVYACEVTHQGLSSPVTKSFNREGC
SEQ ID NO:35	Amino acid sequence of the heavy chain of the humanized antibody VEGF- H988 containing the signal peptide	Amino acid sequence of the heavy chain signal peptide(SEQ ID NO:37):
		MGWSLILLFLVAVATRVLS
		Amino acid sequence of the heavy chain variable region(SEQ ID NO:39):
		QSVQESGPGLVKPSETLSLTCTVSGI DLSSYAISWVRQPPGKGLEYIGYIWN DL FTYYASWAKGRVTISVDTSKNQVDLKL SSVTAADTAVYFCARGTLGDYGGMDP WGQGTLVTVSS
		Amino acid sequence of the human IgG1heavychain constant region(SEQ ID NO:41):
		ASTKGPSVFPLAPSSKSTSGGTAALG CLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVVTVPSSSLGTQTYI CNVNHKPSNTKVDKKVEPKSCDKTHTC PPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDG VEVHNAKTKPREEQYNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKT ISKAKGQPREPQVYTLPPSRDELTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNY KTPPVLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSP GK
SEQ ID NO:36	Amino acid sequence of the light chain of the humanized antibody VEGF-	Amino acid sequence of the light chain signal peptide(SEQ ID NO:38):
		MGWSCILFLVATATGVHS
		Amino acid sequence of the light chain variable region (SEQ ID NO:40):

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	H988 containing the signal peptide	ELQLTQSPSSLSASVGDRVITTCQSS KFLWQGRRLAWYQQKPGKVPKLLIYG ASTLASGVPSRFRKGSGSGTDFTLTISLQ PEDVATYYCAGYKSYDGDVVGFGGGT KVEIK
		Amino acid sequence of the human kappa light chain constant region(SEQ ID NO:42):
		RTVAAPSVFIFPPSDEQLKSGTASVV CLLNNFYPREAKVQWKVDNALQSGNS QESVTEQDSKDYSLSTLTLSKADYE KHKVYACEVTHQGLSSPVTKSFNRGEC
SEQ ID NO:37	Amino acid sequence of the heavy chain signal peptide	MGWSLILLFLVAVATRVLS
SEQ ID NO:38	Amino acid sequence of the light chain signal peptide	MGWSCILFLVATATGVHS
SEQ ID NO:39	Amino acid sequence of the heavy chain variable region of the humanized antibody VEGF-H988	QSVQESGPGLVKPSETLSLTCTVSGI DLSSYAISWVRQPPGKGLEYIGYIWN DL FTYYASWAKGRVTISVDTSKNQVDLKL SSVTAADTAVYFCARGTLGDYGGMDP WGQGTLVTVSS
SEQ ID NO:40	Amino acid sequence of the light chain variable region of humanized antibody VEGF-H988	ELQLTQSPSSLSASVGDRVITTCQSS KFLWQGRRLAWYQQKPGKVPKLLIYG ASTLASGVPSRFRKGSGSGTDFTLTISLQ PEDVATYYCAGYKSYDGDVVGFGGGT KVEIK

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SEQ ID NO:41	Amino acid sequence of the human IgG1 heavy chain constant region	ASTKGPSVFPLAPSSKSTSGGTAALG CLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVVTVPSSSLGTQTYI CNVNHKPSNTKVDKKVEPKSCDKTHTC PPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDG VEVHNAKTKPREEQYNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKT ISKAKGQPREPQVYTLPPSRDELTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNY KTPPVLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSP GK
SEQ ID NO:42	Amino acid sequence of the human kappa light chain constant region	RTVAAPSVFIFPPSDEQLKSGTASVV CLLNNFYPREAKVQWKVDNALQSGNS QESVTEQDSKDSTYSLSSTLTLSKADYE KKHKVYACEVTHQGLSSPVTKSFNRGEC
SEQ ID NO:43	Nucleotide sequence of the humanized antibody VEGF- H988 heavy chain containing the signal peptide	Nucleotide sequence of heavy chain signal peptide(SEQ ID NO:45): ATGGGCTGGTCCCTGATTCTGCTGT TCCTGGTGGCTGTGGCTACCAGGGTGC TGAGT Nucleotide sequence of heavy chain variable region(SEQ ID NO:47): CAGTCTGTCCAGGAGTCTGGACCT GGACTGGTGAAGCCATCTGAGACCCT GTCCCTGACTTGTACTGTGTCTGGCAT TGACCTGTCCTCCTATGCCATCTCCTG GGTGAGACAACCTCCTGGCAAGGGAT TGGAATACATTGGCTACATCTGGAAT GATCTCTTCACCTACTATGCCTCCTGG GCTAAGGGCAGGGTGACCATCTCTGT GGACACCAGCAAGAACCAGGTGGACC

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		TGAAACTGTCCTCTGTGACAGCAGCA GACACAGCAGTCTACTTCTGTGCCAG GGGCACCCTGGGAGACTACGGCGGGA TGGACCCATGGGGACAGGGCACCCTG GTGACAGTGTCCAGC Nucleotide sequence of the human IgG1 heavy chain constant region(SEQ ID NO:49): GCAAGCACCAAGGGCCCATCGGTC TTCCCCCTGGCACCCTCCTCCAAGAGC ACCTCTGGGGGCACAGCGGCCCTGGG CTGCCTGGTCAAGGACTACTTCCCCGA ACCGGTGACGGTGTCTGTGGAATCAG GCGCCCTGACCAGCGGCGTGACACC TTCCCGGCTGTCCTACAGTCCTCAGGA CTCTACTCCCTCAGCAGCGTGGTGACC GTGCCCTCCAGCAGCTTGGGCACCCA GACCTACATCTGCAACGTGAATCACA AGCCCAGCAACACCAAGGTGGACAAG AAAGTTGAGCCCAAATCTTGTGACAA AACTCACACATGCCACCGTGCCAG CACCTGAACTCCTGGGGGGACCGTCA GTCTTCCTCTTCCCCC AAAACCCAAG GACACCCTCATGATCTCCCGGACCCCT GAGGTCACGTGCGTGGTGGTGGACGT GAGCCACGAAGACCCCGAGGTCAAGT TCAACTGGTACGTGGACGGCGTGGAG GTGCATAATGCCAAGACAAAGCCGCG GGAGGAGCAGTACAACAGCACGTACC GTGTGGTCAGCGTCCTCACCGTCCTGC ACCAGGACTGGCTGAATGGCAAGGAG TACAAGTGCAAGGTCTCCAACAAAGC CCTCCCAGCCCCCATCGAGAAAACCA TCTCCAAAGCCAAAGGGCAGCCCCGA
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		GAACCACAGGTGTACACCCTGCCCCC ATCCCGGGATGAGCTGACCAAGAACC AGGTCAGCCTGACCTGCCTGGTCAA GGCTTCTATCCCAGCGACATCGCCGTG GAGTGGGAGAGCAATGGGCAGCCGGA GAACAACACTACAAGACCACGCCTCCCG TGCTGGACTCCGACGGCTCCTTCTTCC TCTACAGCAAGCTCACCGTGGACAAG AGCAGGTGGCAGCAGGGGAACGTCTT CTCATGCTCCGTGATGCATGAGGCTCT GCACAACCACTACACGCAGAAGAGCC TCTCCCTGTCTCCGGGTAAATAA
SEQ ID NO:44	Nucleotide sequence of humanized antibody VEGF- H988 light chain containing signal peptide	Nucleotide sequence of the light chain signal peptide(SEQ ID NO:46): ATGGGCTGGTCCTGTATCATCCTGT TCCTGGTGGCTACAGCCACAGGAGTG CATAGT Nucleotide sequence of the light chain variable region(SEQ ID NO:48): GAACTCCAACCTTACCCAGAGCCCA TCCTCCCTGTCTGCCTCTGTGGGAGAC AGGGTGACCATCACTTGTCAGTCCAG CAAGTTCCTCTGGCAGGGCAGGAGAC TGGCTTGGTATCAACAGAAGCCTGGC AAGGTGCCAAAACCTGCTGATTTATGG AGCCAGCACCCCTGGCATCTGGAGTGC CAAGCAGGTTCAAGGGCTCTGGCTCT GGCACAGACTTCACCCTGACCATCTCC TCCCTCCAACCTGAGGATGTGGCTACC TACTACTGTGCTGGCTACAAGTCCTAT GATGGAGATGTTGTGGGCTTTGGAGG AGGCACCAAGGTGGAGATTAAG Nucleotide sequence of the human kappa light chain constant region(SEQ ID NO:50):

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		CGTACGGTGGCTGCACCATCTGTCT TCATCTTCCC GCCATCTGATGAGCAGT TGAAATCTGGAAGTGCCTCTGTTGTGT GCCTGCTGAATAACTTCTATCCCAGAG AGGCCAAAGTACAGTGGAAGGTGGAT AACGCCCTCCAATCGGGTAACTCCCA GGAGAGTGTACAGAGCAGGACAGCA AGGACAGCACCTACAGCCTCAGCAGC ACCCTGACGCTGAGCAAAGCAGACTA CGAGAAACACAAAGTCTACGCCTGCG AAGTCACCCATCAGGGCCTGAGCTCG CCCGTCACAAAGAGCTTCAACAGGGG AGAGTGTTAA
SEQ ID NO:45	Nucleotide sequence of heavy chain signal peptide	ATGGGCTGGTCCCTGATTCTGCTGT TCCTGGTGGCTGTGGCTACCAGGGTGC TGAGT
SEQ ID NO:46	Nucleotide sequence of the light chain signal peptide	ATGGGCTGGTCCTGTATCATCCTGT TCCTGGTGGCTACAGCCACAGGAGTG CATAGT
SEQ ID NO:47	Nucleotide sequence of the heavy chain variable region of the humanized antibody VEGF- H988	CAGTCTGTCCAGGAGTCTGGACCT GGACTGGTGAAGCCATCTGAGACCCT GTCCCTGACTTGTACTGTGTCTGGCAT TGACCTGTCCTCCTATGCCATCTCCTG GGTGAGACAACCTCCTGGCAAGGGAT TGGAATACATTGGCTACATCTGGAAT GATCTCTTACCTACTATGCCTCCTGG GCTAAGGGCAGGGTGACCATCTCTGT GGACACCAGCAAGAACCAGGTGGACC TGAAACTGTCCTCTGTGACAGCAGCA GACACAGCAGTCTACTTCTGTGCCAG GGGCACCTGGGAGACTACGGCGGGA

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		TGGACCCATGGGGACAGGGCACCCTG GTGACAGTGTCCAGC
SEQ ID NO:48	Nucleotide sequence of the light chain variable region of the humanized antibody VEGF- H988	GAACTCCAACCTTACCCAGAGCCCA TCCTCCCTGTCTGCCTCTGTGGGAGAC AGGGTGACCATCACTTGTCAGTCCAG CAAGTTCCTCTGGCAGGGCAGGAGAC TGGCTTGGTATCAACAGAAGCCTGGC AAGGTGCCAAAAGCTGCTGATTTATGG AGCCAGCACCCCTGGCATCTGGAGTGC CAAGCAGGTTCAAGGGCTCTGGCTCT GGCACAGACTTCACCCTGACCATCTCC TCCCTCCAACCTGAGGATGTGGCTACC TACTACTGTGCTGGCTACAAGTCCTAT GATGGAGATGTTGTGGGCTTTGGAGG AGGCACCAAGGTGGAGATTAAG
SEQ ID NO:49	Nucleotide sequence of the human IgG1heavy chain constant region	GCAAGCACCAAGGGCCCATCGGTC TTCCCCCTGGCACCCCTCCTCCAAGAGC ACCTCTGGGGGGCACAGCGGCCCTGGG CTGCCTGGTCAAGGACTACTTCCCCGA ACCGGTGACGGTGTCGTGGAAGTCAAG GCGCCCTGACCAGCGGCGTGACACC TTCCCGGCTGTCCTACAGTCCTCAGGA CTCTACTCCCTCAGCAGCGTGGTGACC GTGCCCTCCAGCAGCTTGGGCACCCA GACCTACATCTGCAACGTGAATCACA AGCCCAGCAACACCAAGGTGGACAAG AAAGTTGAGCCCAAATCTTGTGACAA AACTCACACATGCCACCGTGCCAG CACCTGAACTCCTGGGGGGACCGTCA GTCTTCCTCTTCCCCCCTAAACCCAAG GACACCCTCATGATCTCCCGGACCCCT GAGGTCACGTGCGTGGTGGTGGACGT GAGCCACGAAGACCCCGAGGTCAAGT TCAACTGGTACGTGGACGGCGTGGAG

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		GTGCATAATGCCAAGACAAAGCCGCG GGAGGAGCAGTACAACAGCACGTACC GTGTGGTCAGCGTCCTCACCGTCCTGC ACCAGGACTGGCTGAATGGCAAGGAG TACAAGTGCAAGGTCTCCAACAAAGC CCTCCCAGCCCCCATCGAGAAAACCA TCTCCAAAGCCAAAGGGCAGCCCCGA GAACCACAGGTGTACACCCTGCCCCC ATCCCGGGATGAGCTGACCAAGAACC AGGTCAGCCTGACCTGCCTGGTCAA GGCTTCTATCCCAGCGACATCGCCGTG GAGTGGGAGAGCAATGGGCAGCCGGA GAACAACACTACAAGACCACGCCTCCCG TGCTGGACTCCGACGGCTCCTTCTTCC TCTACAGCAAGCTCACCGTGGACAAG AGCAGGTGGCAGCAGGGGAACGTCTT CTCATGCTCCGTGATGCATGAGGCTCT GCACAACCACTACACGCAGAAGAGCC TCTCCCTGTCTCCGGGTAAATAA
SEQ ID NO:50	Nucleotide sequence of the human kappa light chain constant region	CGTACGGTGGCTGCACCATCTGTCT TCATCTTCCCGCCATCTGATGAGCAGT TGAAATCTGGAAGTGCCTCTGTTGTGT GCCTGCTGAATAACTTCTATCCCAGAG AGGCCAAAGTACAGTGGAAGGTGGAT AACGCCCTCCAATCGGGTAACTCCCA GGAGAGTGTACAGAGCAGGACAGCA AGGACAGCACCTACAGCCTCAGCAGC ACCCTGACGCTGAGCAAAGCAGACTA CGAGAAACACAAAGTCTACGCCTGCG AAGTCACCCATCAGGGCCTGAGCTCG CCCGTCACAAAGAGCTTCAACAGGGG AGAGTGTTAA
SEQ ID NO:51	Amino acid sequence of the	Amino acid sequence of VEGF165-R988 light chain variable region(SEQ ID NO:12):

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	rabbit antibody scFv which is used in the construction of antibody VEGF165-R988	ELDLTQTPSPVSAAVGGTVTINCQSS QTIYANRRRLAWYQQKPGQPPKLLIYGA STLASGVPSRFKSGSGGTQFTLTISGVQC DDAATYYCAGYKSYDGDDVGFGGGTE VVVK
		linker(SEQ ID NO:52):
		SSGGGGSGGGGGGSSRSS
		Amino acid sequence of VEGF165-R988 heavy chain variable region(SEQ ID NO:11):
		QSVEESGGRLVTPGTPLTLTCTVSGI DLSSYAISWVRQAPGKGLYIGYIWNA GNTYYASWAKGRFTISKSTTTVDLKITS PTTEDTATYFCARGTLGDYNGMDPWGP GTLVTVSS
SEQ ID NO:52	Amino acid sequence of the linker used in the construction of the phage antibody library for the linkage of the rabbit antibody scFv	SSGGGGSGGGGGGSSRSS
SEQ ID NO:53	Nucleotide sequence of the rabbit antibody VEGF165-R988 heavy chain containing the signal peptide	Nucleotide sequence of heavy chain signal peptide(SEQ ID NO:45):
		ATGGGCTGGTCCCTGATTCTGCTGT TCCTGGTGGCTGTGGCTACCAGGGTGC TGAGT
		Nucleotide sequence of heavy chain variable region(SEQ ID NO:7):
		CAGTCGGTGGAGGAGTCCGGGGGT CGCCTGGTAACGCCTGGGACACCCCT GACACTCACCTGCACAGTCTCTGGAAT

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		CGACCTCAGTAGCTATGCAATAAGCT GGGTCCGCCAGGCTCCAGGGAAGGGG CTGGAATACATCGGATACATTTGGAA TGCTGGTAACACCTACTACGCGAGCT GGGCAAAAGGCCGATTCACCATCTCC AAAACCTCGACCACGGTGGATCTGAA AATCACCAGTCCGACAACCGAGGACA CGGCCACCTATTTCTGTGCCAGAGGA ACATTAGGGGACTACAATGGCATGGA CCCCTGGGGCCCAGGGACCCTCGTCA CCGTCTCTTCA
		Nucleotide sequence of the rabbit IgG heavy chain constant region(SEQ ID NO:9):
		GGTCAACCTAAGGCTCCGTCAGTC TTCCCCTGGCCCCCTGCTGCGGGGAC ACACCCAGCTCCACGGTGACCCTGGG CTGCCTGGTCAAAGGCTACCTCCCGG AGCCAGTGACCGTGACCTGGAACCTCG GGCACCCCTACCAATGGGGTACGCAC CTTCCCGTCCGTCCGGCAGTCCTCAGG CCTCTACTCGCTGAGCAGCGTGGTGA GCGTGACCTCAAGCAGCCAGCCCGTC ACCTGCAACGTGGCCCACCCAGCCAC CAACACCAAAGTGGACAAGACCGTTG CGCCCTCGACATGCAGCAAGCCACG TGCCCACCCCCTGAACTCCTGGGGGG ACCGTCTGTCTTCATCTTCCCCC AAA ACCCAAGGACACCCTCATGATCTCAC GCACCCCCGAGGTACATGCGTGGTG GTGGACGTGAGCCAGGATGACCCCGA GGTGCAGTTCACATGGTACATAAACA ACGAGCAGGTGCGCACCGCCCGGCCG CCGCTACGGGAGCAGCAGTTCAACAG CACGATCCGCGTGGTCAGCACCCCTCCC

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		CATCGCGCACCAGGACTGGCTGAGGG GCAAGGAGTTCAAGTGCAAAGTCCAC AACAAGGCACTCCCGGCCCCCATCGA GAAAACCATCTCCAAAGCCAGAGGGC AGCCCCTGGAGCCGAAGGTCTACACC ATGGGCCCTCCCGGGAGGAGCTGAG CAGCAGGTCGGTCAGCCTGACCTGCA TGATCAACGGCTTCTACCCTTCCGACA TCTCGGTGGAGTGGGAGAAGAACGGG AAGGCAGAGGACAACACTACAAGACCAC GCCGGCCGTGCTGGACAGCGACGGCT CCTACTTCCTCTACAGCAAGCTCTCAG TGCCACGAGTGAGTGGCAGCGGGGC GACGTCTTCACCTGCTCCGTGATGCAC GAGGCCTTGCACAACCACTACACGCA GAAGTCCATCTCCCGCTCTCCGGGTAA ATAA
SEQ ID NO:54	Nucleotide sequence of the rabbit antibody VEGF165-R988 light chain containing signal peptide	Nucleotide sequence of the light chain signal peptide(SEQ ID NO:46):
		ATGGGCTGGTCCTGTATCATCCTGT TCCTGGTGGCTACAGCCACAGGAGTG CATAGT
		Nucleotide sequence of the light chain variable region(SEQ ID NO:8):
		GAGCTCGATCTGACCCAGACTCCA TCCCCCGTGTCTGCGGCTGTTGGAGGC ACAGTCACCATCAATTGCCAGTCCAGT CAGACTATTTATGCTAACAGGCGCTTA GCCTGGTATCAACAGAAACCAGGGCA GCCTCCCAAGCTCCTGATCTATGGTGC ATCCACTCTGGCATCTGGGGTCCCATC GCGGTTCAAAGGCAGTGGATCTGGGA CACAGTTCACTCTCACCATCAGCGGCG TGCAGTGTGACGATGCTGCCACTTACT

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		ACTGTGCAGGCTATAAAAGTTATGAT GGTGATGATGTTGGTTTCGGCGGAGG GACCGAGGTGGTCGTCAA
		Nucleotide sequence of the rabbit kappa light chain constant region(SEQ ID NO:10):
		GGGGATCCAGTTGCACCTACTGTC CTCATCTTCCCACCAGCTGCTGATCAG GTGGCAACTGGAACAGTCACCATCGT GTGTGTGGCGAATAAATACTTTCCCGA TGTCACCGTCACCTGGGAGGTGGATG GCACCACCCAAACAACCTGGCATCGAG AACAGTAAAACACCGCAGAATTCTGC AGATTGTACCTACAACCTCAGCAGCA CTCTGACACTGACCAGCACACAGTAC AACAGCCACAAAGAGTACACCTGCAA GGTGACCCAGGGCACGACCTCAGTCG TCCAGAGCTTCAATAGGGGTGACTGTT AA
SEQ ID NO:55	Amino acid sequence of the rabbit antibody VEGF165-R988 heavy chain containing the signal peptide	Amino acid sequence of heavy chain signal peptide(SEQ ID NO:37):
		MGWSLILLFLVAVATRVLS
		Amino acid sequence of heavy chain variable region(SEQ ID NO:11):
		QSVEESGGRLVTPGTPLTLTCTVSGI DLSSYAISWVRQAPGKLEYIGYIWNA GNTYYASWAKGRFTISKSTTTVDLKITS PTTEDTATYFCARGTLGDYNGMDPWGP GTLVTVSS
		Amino acid sequence of the rabbit heavy chain constant region(SEQ ID NO:57):

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		GQPKAPSVFPLAPCCGDTSPSSTVTLG CLVKGYLPEPVTVTWNSGTLTNGVRTF PSVRQSSGLYSLSSVVSVTSSSQPVTCN VAHPATNTKVDKTVAPSTCSKPTCPPPE LLGGPSVFIFPPKPKDTLMISRTPEVTCV VVDVSQDDPEVQFTWYINNEQVRTARP PLREQQFNSTIRVVSTLPIAHQDWLRGK EFKCKVHNKALPAPIEKTISKARGQPLE PKVYTMGPPREELSSRSVSLTCMINGFY PSDISVEWEKNGKAEDNYKTTPAVLDS DGSYFLYSKLSVPTSEWQRGDVFTCSV MHEALHNHYTQKSISRSPGK
SEQ ID NO:56	Amino acid sequence of the rabbit antibody VEGF165-R988 light chain containing signal peptide	Amino acid sequence of the light chain signal peptide(SEQ ID NO:38):
		MGWSCILFLVATATGVHS
		Amino acid sequence of the light chain variable region(SEQ ID NO:12):
		ELDLTQTPSPVSAAVGGTVTINCQSS QTIYANRRRLAWYQQKPGQPPKLLIYGA STLASGVPSRFKGSQSGTQFTLTISGVQC DDAATYYCAGYKSYDGDDVGFGGGTE VVVK
		Amino acid sequence of the rabbit light chain constant region(SEQ ID NO:58)
		GDPVAPTVLIFPPAADQVATGTVTIV CVANKYFPDVTVTWEVDGTTQTTGIEN SKTPQNSADCTYNLSSTLTLTSTQYNH KEYTCKVTQGTTSVVQSFNRGDC
SEQ ID NO:57	Amino acid sequence of the rabbit heavy chain constant region	GQPKAPSVFPLAPCCGDTSPSSTVTLG CLVKGYLPEPVTVTWNSGTLTNGVRTF PSVRQSSGLYSLSSVVSVTSSSQPVTCN VAHPATNTKVDKTVAPSTCSKPTCPPPE LLGGPSVFIFPPKPKDTLMISRTPEVTCV

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		VVDVSQDDPEVQFTWYINNEQVRTARP PLREQQFNSTIRVVSTLPIAHQDWLRGK EFKCKVHNKALPAPIEKTISKARGQPLE PKVYTMGPPREELSSRSVSLTCMINGFY PSDISVEWEKNGKAEDNYKTTPAVLDS DGSYFLYSKLSVPTSEWQRGDVFTCSV MHEALHNHYTQKSISRSPGK
SEQ ID NO:58	Amino acid sequence of the rabbit light chain constant region	GDPVAPTVLIFPPAADQVATGTVTIV CVANKYFPDVTVTWEVDGTTQTTGIEN SKTPQNSADCTYNLSSTLTLTSTQYNH KEYTCKVTQGTTSVVQSFNRGDC

What is claimed are:

1. An isolated anti-VEGF antibody or antigen-binding fragment thereof, comprising

5 a heavy chain variable region having a heavy chain CDR1 region having the amino acid sequence as set forth in SEQ ID NO: 30 and a heavy chain CDR2 region having the amino acid sequence as set forth in SEQ ID NO: 31 and a heavy chain CDR3 region having the amino acid sequence as set forth in SEQ ID NO: 32; and
10 a light chain variable region having a light chain CDR1 region having the amino acid sequence as set forth in SEQ ID NO: 27, a light chain CDR2 region having the amino acid sequence as set forth in SEQ ID NO: 28, and a light chain CDR3 region having the amino acid sequence as set forth in SEQ ID NO: 29.

15 2. The anti-VEGF antibody or antigen-binding fragment thereof according to claim 1, comprising

a heavy chain variable region having the amino acid sequence as set forth in SEQ ID NO: 39, or the amino acid sequences having at least
20 90%, 92%, 95%, 98% or 99% sequence identity to SEQ ID NO: 39; and
a light chain variable region having the amino acid sequence as set forth in SEQ ID NO: 40, or the amino acid sequences having at least 90%, 92%, 95%, 98% or 99% sequence identity to SEQ ID NO: 40.

25 3. The anti-VEGF antibody or antigen-binding fragment thereof according to claim 1 or 2, further comprising

a light chain constant region and a heavy chain constant region.

30 4. The anti-VEGF antibody or antigen binding fragment thereof according to claim 3, wherein

the light chain constant region has the amino acid sequence as set forth in SEQ ID NO: 42, or the amino acid sequences having at least 90%, 92%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 42; and/or

the heavy chain constant region is the IgG1 heavy chain constant region having the amino acid sequence as set forth in SEQ ID NO: 41, or the amino acid sequences having at least 90%, 92%, 95%, 98%, or 99% sequence identity to SEQ ID NO:41.

5. The anti-VEGF antibody or antigen-binding fragment thereof according to any one of claims 1 to 4, wherein said anti-VEGF antibody or antigen-binding fragment thereof is a humanized antibody or a chimeric antibody.

6. The anti-VEGF antibody or antigen-binding fragment thereof according to any one of claims 1 to 5, wherein said anti-VEGF antibody or antigen-binding fragment thereof is a monoclonal antibody.

7. The anti-VEGF antibody or antigen-binding fragment thereof according to any one of claims 1 to 6, wherein said anti-VEGF antibody or antigen-binding fragment thereof is an IgG antibody.

8. The anti-VEGF antibody or antigen-binding fragment thereof according to claim 7, wherein said anti-VEGF antibody or antigen-binding fragment thereof is an IgG1 antibody.

9. The anti-VEGF antibody or antigen-binding fragment thereof according to any one of claims 1 to 8, wherein the binding affinity K_D of said anti-VEGF antibody or antigen-binding fragment thereof to the recombinant human VEGF165 protein is 1-100 pM.

10. The anti-VEGF antibody or antigen-binding fragment thereof according to any one of claims 1 to 8, wherein the binding affinity K_D of said anti-VEGF antibody or antigen-binding fragment thereof to the recombinant human VEGF165 protein is 5-50 pM.

11. The anti-VEGF antibody or antigen-binding fragment thereof according to any one of claims 1 to 8, wherein the binding affinity K_D of said anti-VEGF antibody or antigen-binding fragment thereof to the recombinant human VEGF165 protein is 19.5 pM.

12. The anti-VEGF antibody or antigen-binding fragment thereof according to any one of claims 1-11, wherein said antigen-binding fragment is Fv, Fab, Fab', Fab'-SH, F(ab')₂, Fd fragment, Fd' fragment, single chain antibody molecule or single domain antibody.

13. The anti-VEGF antibody or antigen-binding fragment thereof according to claim 12, wherein the single chain antibody molecule is scFv, di-scFv, tri-scFv, diabody or scFab.

14. An antibody-drug conjugate, comprising the anti-VEGF antibody or antigen-binding fragment thereof according to any one of claims 1-13 and an additional therapeutic agent, wherein said anti-VEGF antibody or antigen-binding fragment thereof is connected with the additional therapeutic agent via a linker.

15. An isolated anti-VEGF antibody or antigen-binding fragment thereof, comprising
a heavy chain variable region having a heavy chain CDR1 region having the amino acid sequence as set forth in SEQ ID NO: 16 and a heavy chain CDR2 region having the amino acid sequence as set forth

in SEQ ID NO: 17 and a heavy chain CDR3 region having the amino acid sequence as set forth in SEQ ID NO: 18; and

5 a light chain variable region having a light chain CDR1 region having the amino acid sequence as set forth in SEQ ID NO: 13, a light chain CDR2 region having the amino acid sequence as set forth in SEQ ID NO: 14, and a light chain CDR3 region having the amino acid sequence as set forth in SEQ ID NO: 15.

10 16. The anti-VEGF antibody or antigen-binding fragment thereof according to claim 15, comprising

a heavy chain constant region having the amino acid sequence as set forth in SEQ ID NO: 25, or the amino acid sequences having at least 90%, 92%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 25; and

15 a light chain variable region having the amino acid sequence as set forth in SEQ ID NO: 26, or the amino acid sequences having at least 90%, 92%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 26.

20 17. The anti-VEGF antibody or antigen-binding fragment thereof according to any one of claims 15-16, wherein said anti-VEGF antibody or antigen-binding fragment thereof is a humanized antibody or a chimeric antibody.

25 18. The anti-VEGF antibody or antigen-binding fragment thereof according to any one of claims 15-17, wherein said anti-VEGF antibody or antigen-binding fragment thereof is a monoclonal antibody.

19. The anti-VEGF antibody or antigen-binding fragment thereof according to any one of claims 15-18,

wherein said antigen-binding fragment is Fv, Fab, Fab', Fab'-SH, F(ab')₂, Fd fragment, Fd' fragment, single chain antibody molecule or single domain antibody.

5 20. The anti-VEGF antibody or antigen-binding fragment thereof according to claim 19, wherein the single chain antibody molecule is scFv, di-scFv, tri-scFv, diabody or scFab.

10 21. A nucleic acid encoding the anti-VEGF antibody or antigen-binding fragment thereof according to any one of claims 1-13, and 15-20.

15 22. The nucleic acid according to claim 21, comprising
the nucleotide sequence as set forth in SEQ ID NO: 7 and the
nucleotide sequence as set forth in SEQ ID NO: 8; or
the nucleotide sequence as set forth in SEQ ID NO: 23 and the
nucleotide sequence as set forth in SEQ ID NO: 24; or
the nucleotide sequence as set forth in SEQ ID NO: 47 and the
nucleotide sequence as set forth in SEQ ID NO: 48.

20 23. An expression vector, comprising the nucleic acid according to claim 21 or 22.

25 24. A host cell, comprising the nucleic acid according to claim 21 or 22 or the expression vector according to claim 23.

30 25. A method for producing the anti-VEGF antibody or antigen-binding fragment thereof according to any one of claims 1-13, and 15-20, comprising culturing the host cells according to claim 24 under conditions suitable for antibody expression, and harvesting the expressed antibodies from the culture medium.

26. A pharmaceutical composition, comprising the anti-VEGF antibody or antigen-binding fragment thereof according to any one of claims 1-13, and 15-20, or the antibody-drug conjugate according to claim 14, or the nucleic acid according to claim 21 or 22, or the expression vector according to claim 23, and a pharmaceutically acceptable carrier.

27. The anti-VEGF antibody or antigen-binding fragment thereof according to any one of claims 1-13, and 15-20 or the antibody-drug conjugate according to claim 14 or the pharmaceutical composition according to claim 26, for use in the treatment of colorectal cancer.

28. A use of the anti-VEGF antibody or antigen-binding fragment thereof according to any one of claims 1-13, and 15-20 or the antibody-drug conjugate according to claim 14 or the pharmaceutical composition according to claim 26 in the preparation of a medicament for the treatment of colorectal cancer.

29. A pharmaceutical combination, comprising the anti-VEGF antibody or antigen-binding fragment thereof according to any one of claims 1-13, and 15-20, or the antibody-drug conjugate according to claim 14, or the pharmaceutical composition according to claim 26, and one or more additional therapeutic agents.

30. A kit, comprising the anti-VEGF antibody or antigen-binding fragment according to any one of claims 1-13, and 15-20, or the antibody-drug conjugate according to claim 14, or the pharmaceutical composition according to claim 26, or the pharmaceutical combination according to claim 29, further comprising a device for administration.

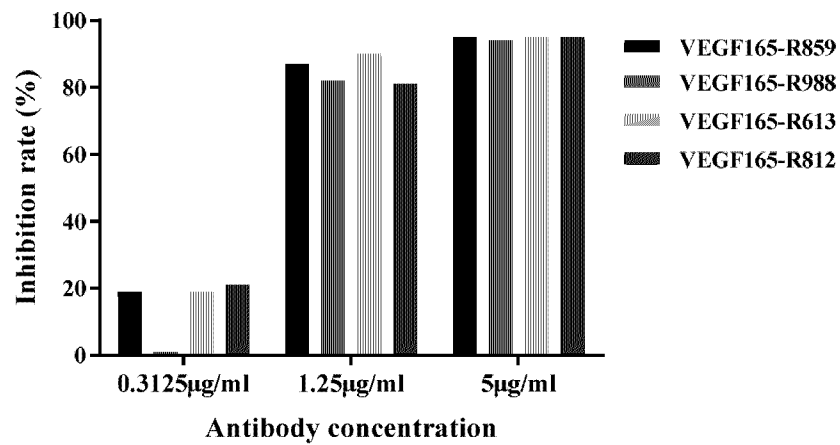


Figure 1. VEGF165 rabbit antibody blocks the binding of VEGF165 to VEGFR2 protein

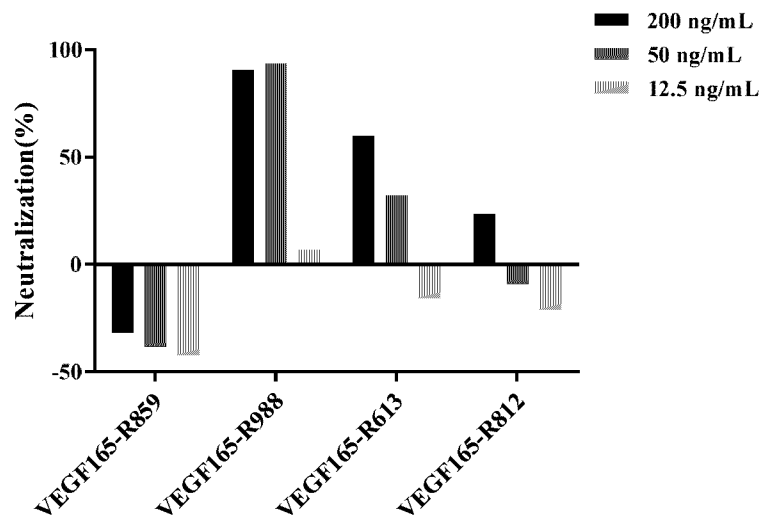


Figure 2. Neutralization rate of VEGF165 rabbit antibodies of VEGF165-induced HUVEC proliferation effect

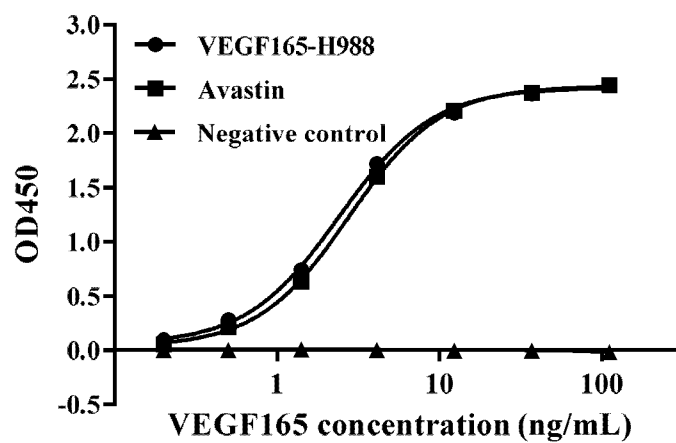


Figure 3. The ELISA assay result of the binding of humanized antibody VEGF165-H988 to VEGF165

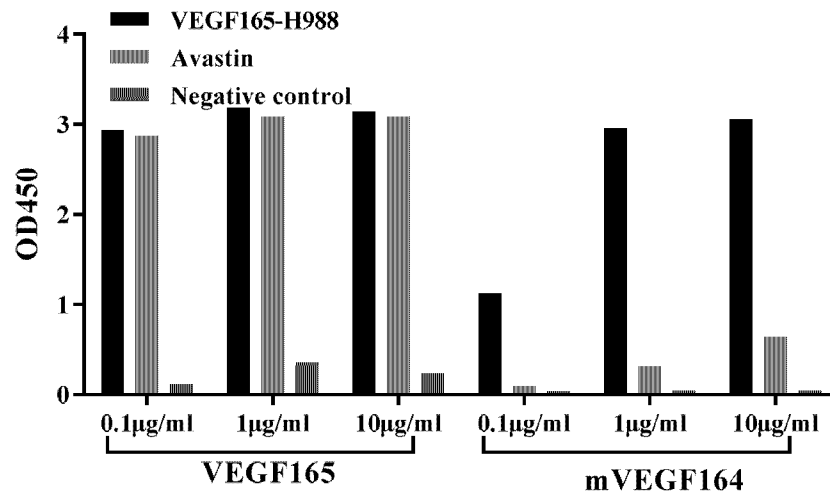


Figure 4. The ELISA assay result of the binding of humanized antibody VEGF165-H988 to mVEGF164

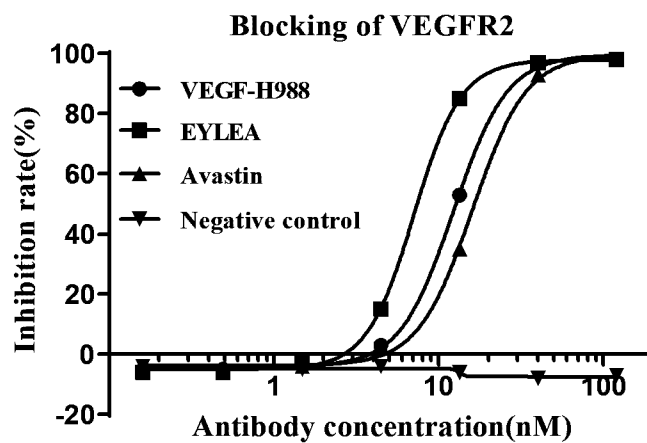


Figure 5. The ELISA assay result of antibody VEGF-H988 blocking the binding of VEGF165 to VEGFR2 protein

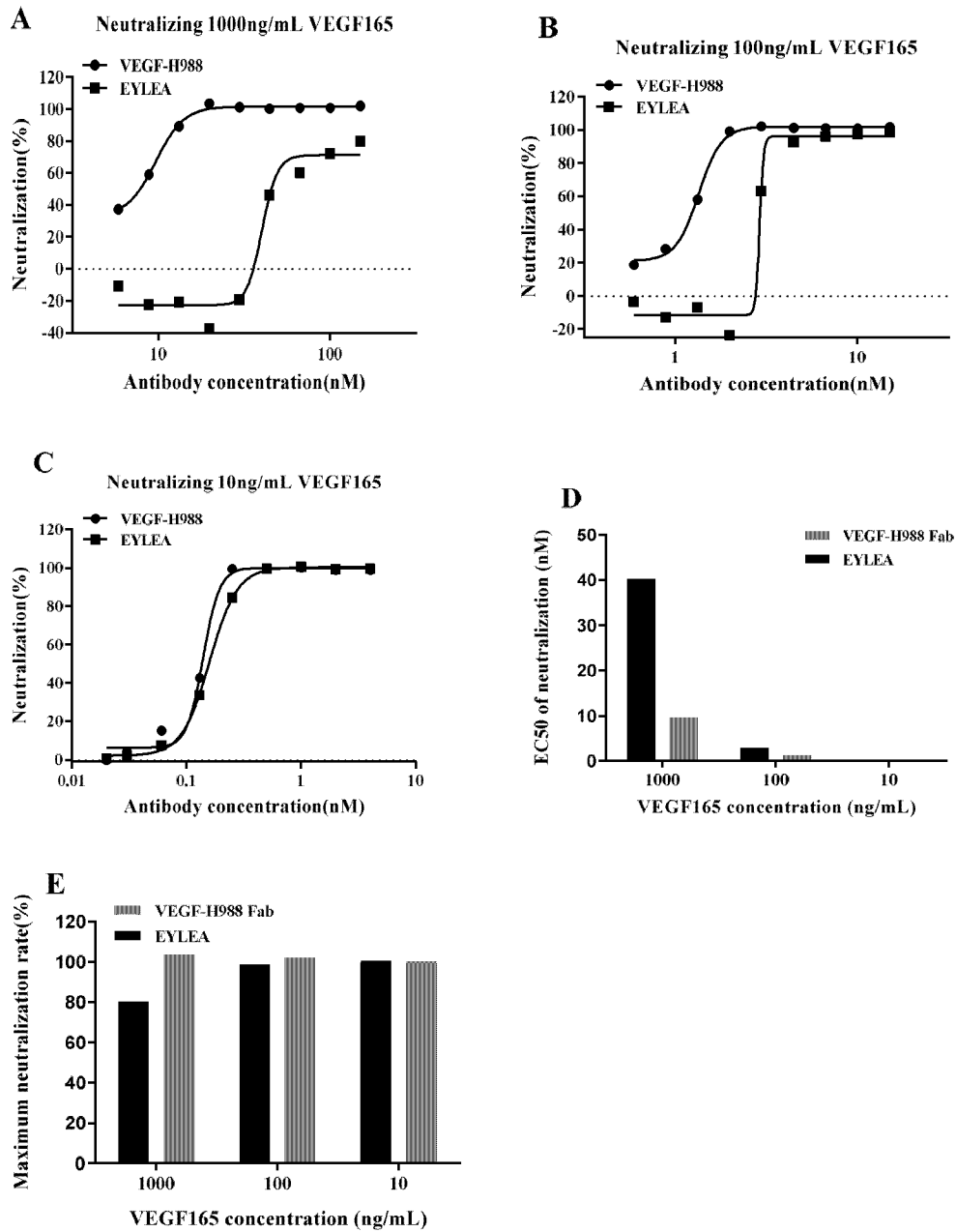


Figure. 6 Comparison of the effects of VEGF-H988 antibody and EYLEA in neutralizing VEGF165 in different concentrations

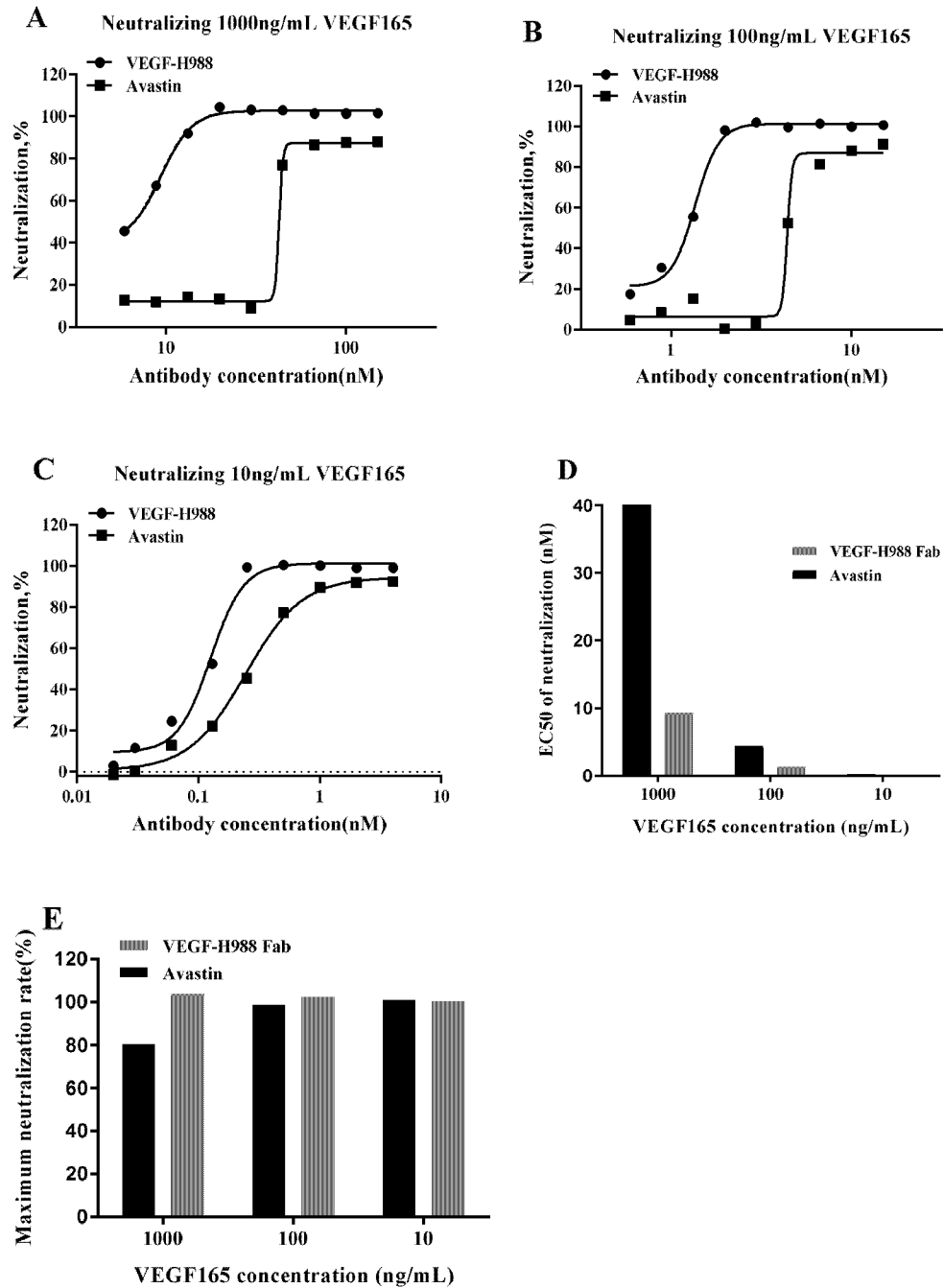


Figure. 7. Comparison of the effects of VEGF-H988 antibody and Avastin in neutralizing VEGF165 in different concentrations

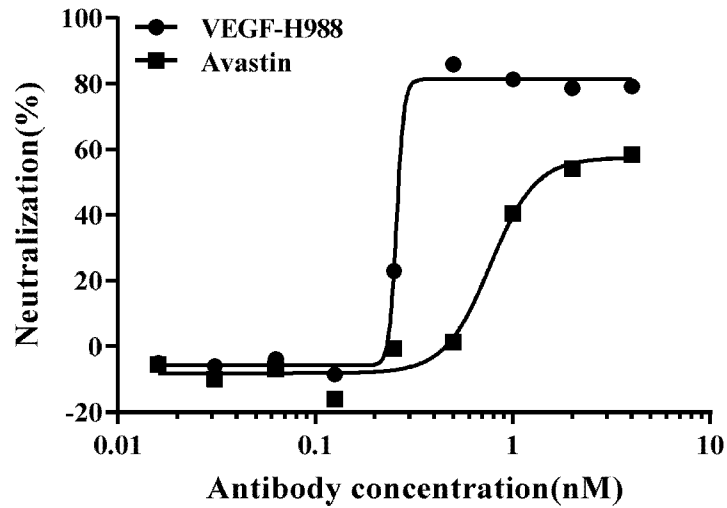


Figure 8. Effects of VEGF-H988 neutralizing of VEGF165, VEGFC and VEGFD - induced proliferation of HUVEC cells

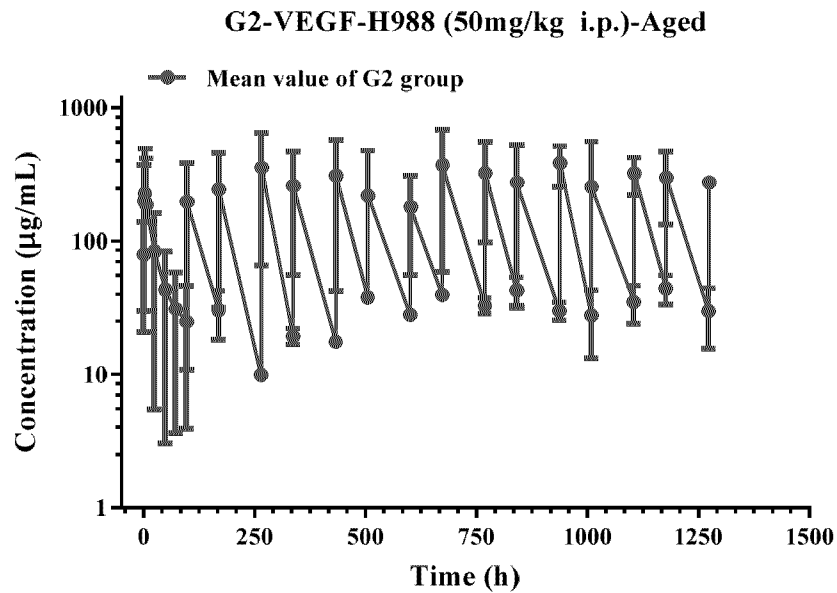


Figure 9. The mean blood concentration-time curve of mice injected with VEGF-H988 (50 mg/kg) intraperitoneally in the G2 group.

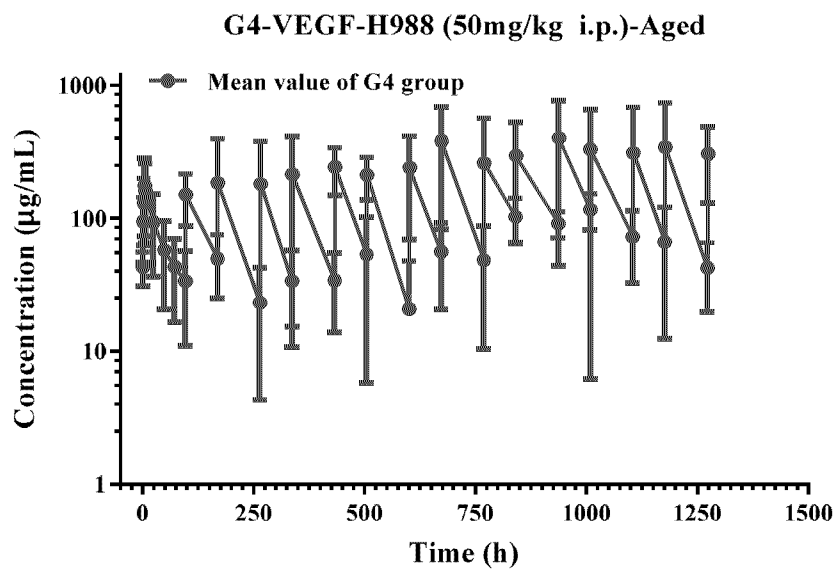


Figure 10. The mean blood concentration-time curve of mice injected with VEGF-H988 (50 mg/kg) intraperitoneally in the G4 group

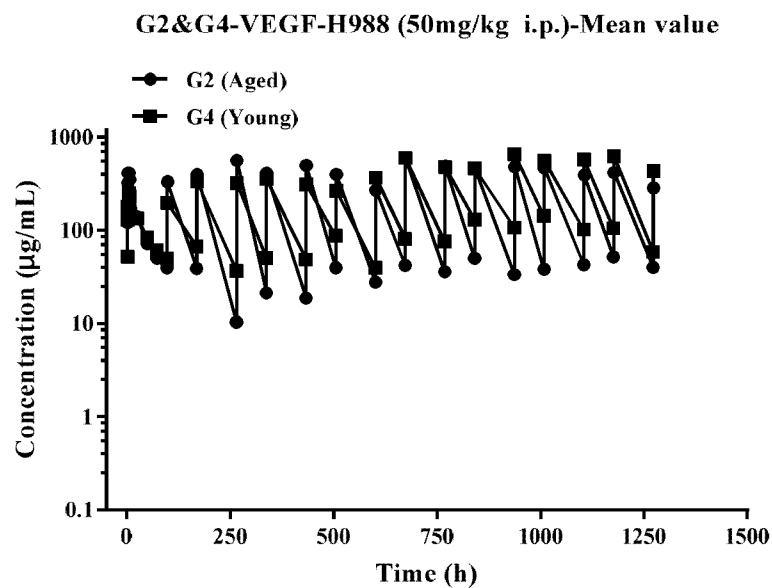


Figure 11. The mean blood concentration-time curve of mice injected with VEGF-H988 (50 mg/kg) intraperitoneally in the G2&G4 groups

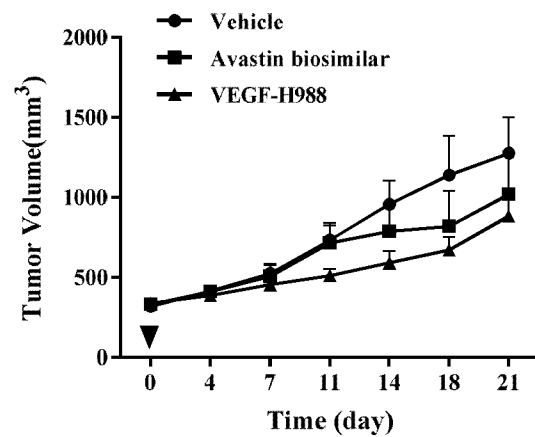
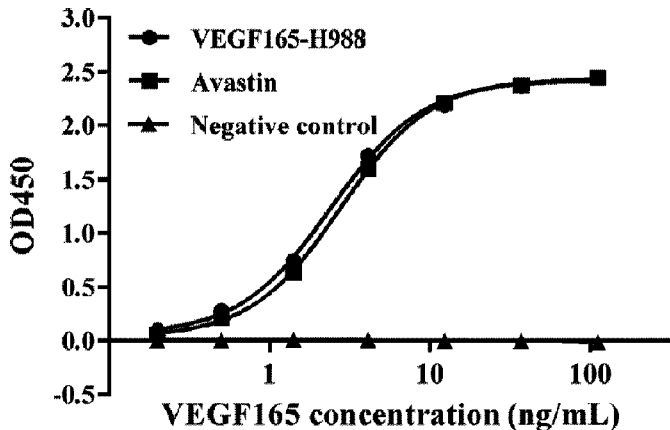


Figure 12. Comparison of the efficacies of VEGF-H988 and the control drugs in the human colorectal cancer cell line HCT-116 xenograft tumor model



The ELISA assay result of the binding of humanized antibody
VEGF165-H988 to VEGF165