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(54) **Title:**
ANTI-VEGF ANTIBODIES AND THEIR USES

(57) **Abstract:**
The present disclosure relates to antibodies directed to vascular endothelial growth factor ("VEGF") and uses of such antibodies, for example to treat diseases associated with the activity and/or overproduction of VEGF.

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[Continued on next page]

(54) **Title**: ANTI-VEGF ANTIBODIES AND THEIR USES

Antibody Chain	CDR No.	Residue	Position in CDR	Kabat No.
Light	1	S	1	24
		A	2	25
		S	3	26
		Q	4	27
		D	5	28
		I	6	29
		S	7	30
		N	8	31
		Y	9	32
		L	10	33
		N	11	34
Light	2	F	1	50
		T	2	51
		S	3	52
		S	4	53
		L	5	54
		H	6	55
		S	7	56
Light	3	Q	1	89
		Q	2	90
		Y	3	91
		S	4	92
		T	5	93
		V	6	94
		P	7	95
		W	8	96
		T	9	97

TABLE 2

(57) **Abstract**: The present disclosure relates to antibodies directed to vascular endothelial growth factor ("VEGF") and uses of such antibodies, for example to treat diseases associated with the activity and/or overproduction of VEGF.

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ANTI-VEGF ANTIBODIES AND THEIR USES

1. CROSS-REFERENCE TO RELATED APPLICATIONS AND SEQUENCE LISTING

[0001] This application claims the benefit under 35 U.S.C. § 119(e) of U.S. provisional application no. 61/218,005, filed June 17, 2009, the contents of which are incorporated herein by reference in their entireties.

[0002] The instant application contains a Sequence Listing which has been submitted via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on June 15, 2010, is named 381493PC.txt and is 141,482 bytes in size.

2. FIELD OF THE INVENTION

[0003] The present invention relates to anti-VEGF antibodies, pharmaceutical compositions comprising anti-VEGF antibodies, and therapeutic uses of such antibodies.

3. BACKGROUND

[0004] Angiogenesis has emerged as attractive therapeutic target due to its implication in a variety of pathological conditions, including tumor growth, proliferative retinopathies, age-related macular degeneration, rheumatoid arthritis (RA), and psoriasis (Folkman *et al.*, 1992, J. Biol. Chem. 267:10931-10934). The first indication of specific molecular angiogenic factors was based on the observation of the strong neovascular response induced by transplanted tumors. It is now known that angiogenesis is essential for the growth of most primary tumors and their subsequent metastasis. Numerous molecules have since been associated with the positive regulation of angiogenesis, including transforming growth factor (TGF)-α, TGF-β, hepatocyte growth factor (HGF), tumor necrosis factor-α, angiogenin, interleukin (IL)-8, and vascular endothelial growth factor (VEGF, also referred to as VEGFA or vascular permeability factor (VPF)) (Ferrara *et al.*, 2003, Nature Medicine 9:669-676).

[0005] The VEGF proteins are important signaling proteins involved in both normal embryonic vasculogenesis (the de novo formation of the embryonic circulatory system) and abnormal angiogenesis (the growth of blood vessels from pre-existing vasculature) (Ferrara *et al.*, 1996, Nature 380:439-442; Dvorak *et al.*, 1995, Am. J. Pathol. 146:1029-1039). VEGF is associated with solid tumors and hematologic malignancies, interocular neovascular syndromes, inflammation and brain edema, and pathology of the female reproductive tract (Ferrara *et al.*, 2003, Nature Medicine 9:669-676). VEGF mRNA is over-expressed in many human tumors, including those of the lung, breast, gastrointestinal tract, kidney, pancreas, and ovary (Berkman *et al.*, 1993, J. Clin. Invest. 91:153-159). Increases in VEGF in the aqueous and vitreous humor of the eyes have been associated with various retinopathies (Aiello *et al.*, 1994, N. Engl. J. Med. 331:1480-1487). Age-related macular degeneration (AMD), a major cause of vision loss in the elderly is due to neovascularization and vascular leakage. The localization of VEGF in the choroidal neovascular membranes in patients affected by AMD has been shown (Lopez *et al.*, 1996, Invest. Ophtalmo.Vis. Sci. 37:855-868).

[0006] The VEGF gene family includes the prototypical member VEGFA, as well as VEGFB, VEGFC, VEGFD, and placental growth factor (PLGF). The human VEGFA gene is organized as eight exons separated by seven introns. At least six different isoforms of VEGF exist, $VEGF_{121}$, $VEGF_{145}$, $VEGF_{162}$, $VEGF_{165}$, $VEGF_{165b}$, $VEGF_{183}$, $VEGF_{189}$, and $VEGF_{206}$, where the subscripts refer to the number of amino acids remaining after signal cleavage. Native VEGF is a 45kDa homodimeric heparin-binding glycoprotein (Ferrara *et al.*, 2003, Nature Medicine 9:669-676). VEGF (specifically VEGFA) binds to two related receptor tyrosine kinases, VEGFR-1 (also referred to as Flt-1) and VEGFR-2 (also referred to as Flk-1 or kinase domain region (KDR) or CD309). Each receptor has seven extracellular and one transmembrane region. VEGF also binds to the neuropilins NRP1 (also referred to as vascular endothelial cell growth factor 165 receptor (VEGF165R) or CD304) and NRP2 also referred to as vascular endothelial cell growth factor 165 receptor 2 (VEGF165R2)).

[0007] Given its central role in regulating angiogenesis, VEGF provides an attractive target for therapeutic intervention. Indeed, a variety of therapeutic strategies aimed at blocking VEGF or its receptor signaling system are currently being developed for the treatment of neoplastic diseases. The anti-VEGF antibody bevacizumab, also referred to as rhuMAb VEGF or Avastin®, is a recombinant humanized anti-VEGF monoclonal antibody created and marketed by Genentech (Presta *et al.*, 1997, Cancer Res. 57:4593-4599). In order to construct bevacizumab the complementarity-determining regions (CDRs) of the murine anti-VEGF monoclonal antibody A.4.6.1 were grafted onto human frameworks and an IgG constant region. Additional mutations outside the CDRs were then introduced into the molecule to improve binding, affording an antibody in which ~93% of the amino acid sequence is derived from human IgG_1 and ~7% of the sequence is derived from the murine antibody A.4.6.1. Bevacizumab has a molecular mass of about 149,000 Daltons and is glycosylated.

[0008] Ranibizumab is an affinity maturated Fab fragment derived from bevacizumab. Ranibizumab has a higher affinity for VEGF and also is smaller in size, allowing it to better penetrate the retina, and thus treat the ocular neovascularization associated with AMD (Lien and Lowman, In: Chernajovsky, 2008, Therapeutic Antibodies. Handbook of Experimental Pharmacology 181, Springer-Verlag, Berlin Heidelberg 131-150). Ranibizumab was developed and is marketed by Genentech under the trade name Lucentis®.

[0009] Treatment of cancer patients with a regimen that includes Avastin® can result in side effects including hypertension, proteinuria, thromboembolic events, bleeding and cardiac toxicity (Blowers & Hall, 2009, Br. J. Nurs. 18(6):351-6, 358). Also, despite being a humanized antibody, bevacizumab can elicit an immune response when administered to humans. Such an immune response may result in an immune complex-mediated clearance of the antibodies or fragments from the circulation, and make repeated administration unsuitable for therapy, thereby reducing the therapeutic benefit to the patient and limiting the re-administration of the antibody.

[0010] Accordingly, there is a need to provide improved anti-VEGF antibodies or fragments that overcome one or more of these problems, for example, by generating variants with higher affinity than bevacizumab that can be administered at reduced dosages, or variants with reduced immunogenicity and other side-effects as compared to bevacizumab.

[0011] Citation or identification of any reference in Section 3 or in any other section of this application shall not be construed as an admission that such reference is available as prior art to the present disclosure.

4. SUMMARY

[0012] The present disclosure relates to variants of the anti-VEGF antibody bevacizumab with reduced immunogenicity and/or improved affinity towards VEGF as compared to bevacizumab or ranibizumab. Bevacizumab has three heavy chain CDRs, referred to herein (in amino- to carboxy-terminal order) as CDR-H1, CDR-H2, and CDR-H3, and three light chain CDRs, referred to herein (in amino- to carboxy-terminal order) as CDR-L1, CDR-L2, and CDR-L3. The sequences of the bevacizumab CDRs are shown in Figures 1A and 1B, and their numbering is set forth in Table 1 (for heavy chain CDRs) and Table 2 (for light chain CDRs). A related antibody, ranibizumab, was generated by affinity maturation of bevacizumab. Ranibizumab has identical CDR-L1, CDR-L2, CDR-L3 and CDR-H2 sequences to bevacizumab, but varies in its CDR-H1 and CDR-H3 sequences from those of bevacizumab. The heavy and light chain sequences of ranibizumab are shown in Figure 1C, and the CDRs are set forth in Figure 1D.

[0013] The antibodies of the disclosure generally have at least one amino acid substitution in at least one heavy chain CDR as compared to bevacizumab and ranibizumab.

[0014] In certain aspects, the anti-VEGF antibodies include at least one substitution as compared to bevacizumab or ranibizumab selected from N31F in CDR-H1; K64S in CDR-H2; K64Q in CDR-H2; Y53F in CDR-H2; H97E in CDR-H3; H97D in CDR-H3; H97P in CDR-H3; Y98F in CDR-H3; Y99E in CDR-H3; Y99D in CDR-H3; S100aG in CDR-H3, and T51A in CDR-L2. In other aspects, the anti-VEGF antibodies include at least one substitution selected from Tables 8 and 9. Additional mutations that can be incorporated into the improved affinity variant antibodies can be candidate deimmunizing substitutions, such as those described in Table 6, as well as other mutations, *e.g.*, substitutions, that do not destroy the ability of the antibodies to bind to VEGF, including but not limited to the mutations described in Tables 10 and 11, or known mutations, such as the mutations described in Tables 12-1 to 12-9 and 13. Yet further mutations that can be incorporated include but are not limited to the mutations described in Tables 14-16.

[0015] In specific embodiments, the anti-VEGF antibodies of the disclosure include a combination of substitutions selected from Table 7, and optionally one or more additional mutations, *e.g.*, candidate deimmunizing substitutions, such as those described in Table 6, as well as other mutations, *e.g.*, substitutions, that do not destroy the ability of the antibodies to bind to VEGF, including but not limited to the mutations described in Tables 10 and 11, or known mutations, such as the mutations described in Tables 12-1 to 12-9 and 13. Yet further mutations that can be incorporated into the anti-VEGF antibodies of the disclosure include but are not limited to the mutations described in Tables 14-16.

[0016] In other embodiments, the anti-VEGF antibodies of the disclosure include one or more of the following CDR substitutions: K64S (CDR-H2), K64Q (CDR-H2), Y53F and K64Q (CDR-H2), H97E and Y98F (CDR-H3), or T51A (CDR-L2). The anti-VEGF antibodies can also optionally include one or more additional mutations or combinations of mutations selected from one or more of Tables 6, 7, 8, 9, 10, 11, 12-1 to 12-9, or 13-16.

[0017] Further CDR substitutions can include N31F (CDR-H1), H97E (CDR-H3), H97D (CDR-H3), H97P (CDR-H3), Y99E (CDR-H3), Y99D (CDR-H3), S100aG (CDR-H3) wherein position 3 in CDR-H3 optionally is not tyrosine, T28P, N31F, N31G and N31M (CDR-H1), H97A, H97Q, H97S, H97T, S100aD, S100aE, and S100Av (CDR-H3), T30W, T30R or T30Q (CDR-H1), Y53F, T58F, A61G, A61K, A61R, A61H, A61Y, K64G, K64E, R65L, R65T, R65A, R65E, and R65D (CDR-H2), and Y98F and Y100eF (CDR-H3). The CDRs optionally contain one or more additional mutations or combinations of mutations selected from one or more of Tables 6, 7, 8, 9, 10, 11, 12-1 to 12-9 and 13.

[0018] Yet further substitutions can include heavy chain CDR substitutions including a combination of substitutions selected from: (a) N31F in CDR-H1, H97D in CDR-H3, Y99D in CDR-H3, and S100aG in CDR-H3; (b) N31F in CDR-H1, H97P in CDR-H3, Y99D in CDR-H3, and S100aG in CDR-H3; (c) N31F in CDR-H1, H97P in CDR-H3, and Y99E in CDR-H3; (d) N31F in CDR-H1, H97E in CDR-H3, and Y99E in CDR-H3; (e) N31F in CDR-H1, H97D in CDR-H3, and Y99E in CDR-H3; (f) N31F in CDR-H1, H97E in CDR-H3, Y99D in CDR-H3, and S100aG in CDR-H3; (g) N31F in CDR-H1, Y99D in CDR-H3, and S100aG in CDR-H3; (h) N31F in CDR-H1, H97P in CDR-H3, and Y99D in CDR-H3; (i) N31F in CDR-H1, H97D in CDR-H3, and S100aG in CDR-H3; (j) N31F in CDR-H1 and S100aG in CDR-H3; or (k) N31F in CDR-H1, H97P in

CDR-H3, and S100aG in CDR-H3. Further optional substitutions can include one or more additional mutations or combinations of mutations selected from one or more of Tables 6, 7, 8, 9, 10, 11, 12-1 to 12-9 and 13.

[0019] Still further heavy chain substitutions can include at least one substitution selected from A61F in CDR-H2, A61E in CDR-H2, A61D in CDR-H2, D62L in CDR-H2, D62G in CDR-H2, D62Q in CDR-H2, D62T in CDR-H2, D62K in CDR-H2, D62R in CDR-H2, D62E in CDR-H2, D62H in CDR-H2, K64S in CDR-H2, K64V in CDR-H2, K64Q in CDR-H2, R65V in CDR-H2, R65F in CDR-H2, R65H in CDR-H2, R65N in CDR-H2, R65S in CDR-H2, R65Q in CDR-H2, R65K in CDR-H2, R65I in CDR-H2, and Y98H in CDR-H3. Optionally, one or more additional mutations or combinations of mutations can be included as selected from one or more of Tables 7, 8, 9, 10, 11, 12-1 to 12-9 and 13.

[0020] In certain aspects, the antibodies of the disclosure have VH and VL sequences having at least 80% sequence identity (and in certain embodiments, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% sequence identity) to the VH and VL sequences of bevacizumab or ranibizumab, and include at least one amino acid substitution in at least one CDR as compared to bevacizumab or ranibizumab. In other aspects, the antibodies of the disclosure have VH and VL sequences having at least 80% sequence identity (and in certain embodiments, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% sequence identity) to the VH and VL sequences of bevacizumab or ranibizumab, and include at least one amino acid substitution in at least one framework region as compared to bevacizumab or ranibizumab. In specific embodiments, the percentage sequence identity for the heavy chain and the light chain compared to the VH and VL sequences of bevacizumab or ranibizumab is independently selected from at least 80%, at least 85%, at least 90%, at least 95% sequence identity, or at least 99% sequence identity. In certain aspects, the antibodies of the disclosure have VH and/or VL sequences having at least 95%, at least 98% or at least 99% sequence identity to the VH and/or VL sequences of bevacizumab or ranibizumab.

[0021] In certain aspects, the antibodies of the disclosure have up to 17 amino acid substitutions in their CDRs as compared to bevacizumab or ranibizumab. Variant antibodies with 17 amino acid substitutions that maintain their target binding capability have been generated by Bostrom *et al.*, 2009, Science 323:1610-14.

[0022] In specific embodiments, an anti-VEGF antibody of the disclosure has, independently:

- up to one, up to two, up to three, up to four, up to five, up to six, up to seven, up to eight, up to nine or up to ten CDR-H1 substitutions as compared to the corresponding CDR of bevacizumab or of ranibizumab;
- up to one, up to two, up to three, up to four, up to five, up to six, up to seven, up to eight, up to nine, up to ten, up to eleven, up to twelve, up to thirteen, up to fourteen, up to fifteen, up to sixteen or up to seventeen CDR-H2 substitutions as compared to the corresponding CDR of bevacizumab or of ranibizumab;
- up to one, up to two, up to three, up to four, up to five, up to six, up to seven, up to eight, up to nine, up to ten, up to eleven, up to twelve, up to thirteen or up to fourteen CDR-H3 substitutions as compared to the corresponding CDR of bevacizumab or of ranibizumab;
- up to one, up to two, up to three, up to four, up to five, up to six, up to seven, up to eight, up to nine, up to ten or up to eleven CDR-L1 substitutions as compared to the corresponding CDR of bevacizumab or of ranibizumab;
- up to one, up to two, up to three, up to four, up to five, up to six or up to seven CDR-L2 substitutions as compared to the corresponding CDR of bevacizumab or of ranibizumab; and
- up to one, up to two, up to three, up to four, up to five, up to six, up to seven, up to eight or up to nine CDR-L3 substitutions as compared to the corresponding CDR of bevacizumab or of ranibizumab.

[0023] The present disclosure further provides pharmaceutical compositions comprising modified anti-VEGF antibodies. In some aspects, the pharmaceutical compositions have increased affinity to VEGF and/or reduced immunogenicity as compared to bevacizumab or ranibizumab.

[0024] Nucleic acids comprising nucleotide sequences encoding the anti-VEGF antibodies of the disclosure are provided herein, as are vectors comprising the nucleic acids. Additionally, prokaryotic and eukaryotic host cells transformed with a vector comprising a nucleotide sequence encoding an anti-VEGF antibody are provided herein, as well as eukaryotic (such as mammalian) host cells engineered to express the nucleotide sequences. Methods of producing anti-VEGF antibodies by culturing host cells are also provided.

[0025] The anti-VEGF antibodies of the disclosure are useful in the treatment of cancers (*e.g.*, colon carcinoma, rectal carcinoma, non-small cell lung cancer, and breast cancer), retinal diseases (*e.g.*, age-related macular degeneration ("AMD")), and immune disorders (*e.g.*, rheumatoid arthritis).

[0026] In certain aspects, the anti-VEGF antibodies of the disclosure can be used in reduced dosages as compared to bevacizumab or ranibizumab, *e.g.,* at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80% or at least 90% lower dosages.

[0027] It should be noted that the indefinite articles "a" and "an" and the definite article "the" are used in the present application, as is common in patent applications, to mean one or more unless the context clearly dictates otherwise. Further, the term "or" is used in the present application, as is common in patent applications, to mean the disjunctive "or" or the conjunctive "and."

[0028] All publications mentioned in this specification are herein incorporated by reference. Any discussion of documents, acts, materials, devices, articles or the like that has been included in this specification is solely for the purpose of providing a context for the present disclosure. It is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present disclosure as it existed anywhere before the priority date of this application.

[0029] The features and advantages of the disclosure will become further apparent from the following detailed description of embodiments thereof.

5. BRIEF DESCRIPTION OF THE TABLES AND FIGURES

[0030] **Table 1** shows the numbering of the amino acids in the heavy chain CDRs of bevacizumab. CDRs 1-3 are disclosed as SEQ ID NOS:3-5, respectively.

[0031] **Table 2** shows the numbering of the amino acids in the light chain CDRs of bevacizumab. CDRs 1-3 are disclosed as SEQ ID NOS:6-8, respectively.

[0032] **Table 3** shows bevacizumab VL peptides that were tested for immunogenicity.

[0033] **Table 4** shows bevacizumab VH peptides that were tested for immunogenicity.

[0034] **Table 5** shows identified $CD4^+$ T cell epitope regions in bevacizumab. CDR regions are underlined.

[0035] **Table 6** shows candidate mutations in CDR-H2 and CDR-H3 for lowering immunogenicity of bevacizumab. The numbering of the amino acids in Table 6 corresponds to Kabat numbering in the bevacizumab heavy chain.

[0036] **Table 7** shows heavy chain CDR amino acid substitutions in bevacizumab resulting improved K_D as analyzed by surface plasmon resonance. Δ k_{on} refers to fold improvement in k_{on} (mutant/WT). Δ k_{off} refers to fold improvement in k_{off} (WT/mutant). Δ K_D refers to the improvement in the K_D in the mutant relative to wild type. The numbering of the amino acids in Table 7 corresponds to Kabat numbering in the bevacizumab heavy chain.

[0037] **Table 8** shows mutations in the bevacizumab heavy chain CDRs that preliminary binding studies indicate increase the affinity towards VEGF (data not shown). The numbering of the amino acids in Table 8 corresponds to Kabat numbering in the bevacizumab heavy chain.

[0038] **Table 9** shows mutations in the bevacizumab heavy chain CDRs that preliminary studies indicate increase the affinity towards VEGF (data not shown). The numbering of the amino acids in Table 9 corresponds to Kabat numbering in the bevacizumab heavy chain.

[0039] **Table 10** shows mutations in the bevacizumab heavy chain CDRs that do not impact binding and can be incorporated into the antibodies of the disclosure. The numbering of the amino acids in Table 10 corresponds to Kabat numbering in the bevacizumab heavy chain.

[0040] **Table 11** shows mutations in the bevacizumab light chain CDRs that do not impact binding and can be incorporated into the antibodies of the disclosure. The numbering of the amino acids in Table 11 corresponds to Kabat numbering in the bevacizumab light chain.

[0041] Tables 12-1 to 12-9 show known mutations in bevacizumab heavy chain CDRs that can be incorporated into the antibodies of the disclosure. Each row in Tables 12-1 to 12-9 includes a distinct known variant. For each variant, the known CDR sequences are shaded. The sequence identifiers for each variant identified in Tables 12-1 to 12-9 are set forth in Tables 20-1 to 20-9, respectively. The CDR-H1 column provides a partial sequence of CDR-H1. The final asparagine of CDR-H1 is not shown. This partial sequence corresponds to SEQ ID NO:411. Although known mutations in CDR-H1 are shown in the context of this partial sequence, it is noted that the mutations exist in the context of the full length CDR.

[0042] Table 13 shows known mutations in bevacizumab light chain CDRs that can be incorporated into the antibodies of the disclosure. Each row in Table 13 includes a distinct known variant. For each variant, the known CDR sequences are shaded. The sequence identifiers for each variant identified in Table 13 is set forth in Table 20-10.

[0043] Table 14 shows bevacizumab CDR2 VH peptides that were tested for immunogenicity, wherein residues unchanged from SEQ ID NO:62 are indicated by a blank box. CD4+ T cell assay results are also provided.

[0044] Table 15 shows bevacizumab CDR3 VH peptides that were tested for immunogenicity, wherein residues unchanged from SEQ ID NO:74 are indicated by a blank box. CD4+ T cell assay results are also provided.

[0045] Table 16 shows bevacizumab CDR2 VL peptides that were tested for immunogenicity, wherein residues unchanged from SEQ ID NO:25 are indicated by a blank box. CD4+ T cell assay results are also provided.

[0046] Table 17 shows selected epitope modifications for the three CD4+ T cell epitopes in bevacizumab.

[0047] Table 18 shows single variable region mutants and their associated mean fluorescence intensity (MFI) score.

[0048] Table 19 shows combined variable region mutants and their associated EC_{50}.

[0049] **Tables 20-1** to **20-10** show the SEQ ID NOS, where known, corresponding to the CDRs of the bevacizumab variants listed in Tables 12-1 to 12-9 and Table 13, respectively. N/A indicates an unknown CDR sequence.

[0050] **Figures 1A-1D**. **Figure 1A** shows the amino acid sequences of the bevacizumab heavy and light chain variable regions, SEQ ID NO:1 and SEQ ID NO:2, respectively, with CDR regions in bold, underlined text. **Figure 1B** shows the CDR sequences and corresponding sequence identifiers of bevacizumab. **Figure 1C** shows the amino acid sequences of the ranibizumab heavy and light chains, SEQ ID NO:9 and SEQ ID NO:10, respectively, with CDR regions in bold, underlined text. **Figure 1D** shows the CDR sequences and corresponding sequence identifiers of ranibizumab.

[0051] **Figures 2A-2B** show bevacizumab VL peptide responses. Figure 2A shows percent of donor responses to each VL peptide with a stimulation index of 2.95 or greater. N = 99 donors. Figure 2B shows the average stimulation index for all 99 donors for each peptide plus or minus standard error.

[0052] **Figures 3A-3B** show bevacizumab VH peptide responses. Figure 3A shows percent of donor responses to each VH peptide with a stimulation index of 2.95 or greater. N = 99 donors. Figure 3B shows the average stimulation index for all 99 donors for each peptide plus or minus standard error.

[0053] **Figures 4A-4C** show CD4+ T cell responses to mutant bevacizumab epitope peptides. Average responses to the unmodified parent epitope sequences are indicated with open marks. Large circles indicate selected changes referred to in **Table 17**. **Figure 4A** is directed to VH CDR2 peptides; **Figure 4B** is directed to VH CDR3 peptides; and **Figure 4C** is directed to VL CDR2 peptides.

6. DETAILED DESCRIPTION

6.1 ANTI-VEGF ANTIBODIES

[0054] The present disclosure provides anti-VEGF antibodies. Unless indicated otherwise, the term "antibody" (Ab) refers to an immunoglobulin molecule that specifically binds to, or is immunologically reactive with, a particular antigen, and includes polyclonal, monoclonal, genetically engineered and otherwise modified forms of antibodies, including but not limited to chimeric antibodies, humanized antibodies, heteroconjugate antibodies (*e.g.*, bispecific antibodies, diabodies, triabodies, and tetrabodies), and antigen binding fragments of antibodies, including *e.g.*, Fab', $F(ab')_2$, Fab, Fv, rIgG, and scFv fragments. Moreover, unless otherwise indicated, the term "monoclonal antibody" (mAb) is meant to include both intact molecules, as well as, antibody fragments (such as, for example, Fab and $F(ab')_2$ fragments) which are capable of specifically binding to a protein. Fab and $F(ab')_2$ fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation of the animal, and may have less non-specific tissue binding than an intact antibody (Wahl *et al.,* 1983, J. Nucl. Med. 24:316).

[0055] The term "scFv" refers to a single chain Fv antibody in which the variable domains of the heavy chain and the light chain from a traditional antibody have been joined to form one chain.

[0056] References to "VH" refer to the variable region of an immunoglobulin heavy chain of an antibody, including the heavy chain of an Fv, scFv, or Fab. References to "VL" refer to the variable region of an immunoglobulin light chain, including the light chain of an Fv, scFv, dsFv or Fab. Antibodies (Abs) and immunoglobulins (Igs) are glycoproteins having the same structural characteristics. While antibodies exhibit binding specificity to a specific target, immunoglobulins include both antibodies and other antibody-like molecules which lack target specificity. Native antibodies and immunoglobulins are usually heterotetrameric glycoproteins of about 150,000 Daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each heavy chain has at the amino terminus a variable domain (VH) followed by a number of constant domains. Each light chain has a variable domain at the amino terminus (VL) and a constant domain at the carboxy terminus.

[0057] The anti-VEGF antibodies of the disclosure bind to human VEGF and inhibit VEGF receptor activity in a cell.

[0058] The anti-VEGF antibodies of the disclosure contain complementarity determining regions (CDRs) that are related in sequence to the CDRs of the antibody bevacizumab (also known as Avastin®) and/or ranibizumab (also known as Lucentis®).

[0059] CDRs are also known as hypervariable regions both in the light chain and the heavy chain variable domains. The more highly conserved portions of variable domains are called the framework (FR). As is known in the art, the amino acid position/boundary delineating a hypervariable region of an antibody can vary, depending on the context and the various definitions known in the art. Some positions within a variable domain may be viewed as hybrid hypervariable positions in that these positions can be deemed to be within a hypervariable region under one set of criteria while being deemed to be outside a hypervariable region under a different set of criteria. One or more of these positions can also be found in extended hypervariable regions. The disclosure provides antibodies comprising modifications in these hybrid hypervariable positions. The variable domains of native heavy and light chains each comprise four FR regions, largely by adopting a β-sheet configuration, connected by three CDRs, which form loops connecting, and in some cases forming part of, the β-sheet structure. The CDRs in each chain are held together in close proximity by the FR regions in the order FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4 and, with the CDRs from the other chain, contribute to the formation of the target binding site of antibodies (see Kabat *et al.,* Sequences of Proteins of Immunological Interest (National Institute of Health, Bethesda, Md. 1987). As used herein, numbering of immunoglobulin amino acid residues is done according to the immunoglobulin amino acid residue numbering system of Kabat *et al.,* unless otherwise indicated.

[0060] The sequences of the heavy and light chain variable regions of bevacizumab are represented by SEQ ID NO:1 and SEQ ID NO:2, respectively. The sequences of the heavy and light chain variable regions are also depicted in Figure 1A. The sequences of the CDRs of bevacizumab, and their corresponding identifiers, are presented in Figure 1B. Any nucleotide sequences encoding SEQ ID NO:1 or SEQ ID NO:2 can be used in the compositions and methods of the present disclosure.

[0061] The sequences of the heavy and light chains of ranibizumab are represented by SEQ ID NO:9 and SEQ ID NO:10, respectively. The sequences of the heavy and light chains are also depicted in Figure 1C. The sequences of the CDRs of ranibizumab, and their corresponding identifiers, are presented in Figure 1D. Any nucleotide sequences encoding SEQ ID NO:9 or SEQ ID NO:10 can be used in the compositions and methods of the present disclosure.

[0062] The present disclosure further provides anti-VEGF antibody fragments comprising CDR sequences that are related to the CDR sequences of bevacizumab and ranibizumab. The term "antibody fragment" refers to a portion of a full-length antibody, generally the target binding or variable region. Examples of antibody fragments include Fab, Fab', $F(ab')_2$ and Fv fragments. An "Fv" fragment is the minimum antibody fragment which contains a complete target recognition and binding site. This region consists of a dimer of one heavy and one light chain variable domain in a tight, non-covalent association (VH–VL dimer). It is in this configuration that the three CDRs of each variable domain interact to define a target binding site on the surface of the VH -VL dimer. Often, the six CDRs confer target binding specificity to the antibody. However, in some instances even a single variable domain (or half of an Fv comprising only three CDRs specific for a target) can have the ability to recognize and bind target. "Single-chain Fv" or "scFv" antibody fragments comprise the VH and VL domains of an antibody in a single polypeptide chain. Generally, the Fv polypeptide further comprises a polypeptide linker between the VH and VL domain which enables the scFv to form the desired structure for target binding. "Single domain antibodies" are composed of a single VH or VL domains which exhibit sufficient affinity to the target. In a specific embodiment, the single domain antibody is a camelid antibody (see, *e.g.*, Riechmann, 1999, Journal of Immunological Methods 231:25–38).

[0063] The Fab fragment contains the constant domain of the light chain and the first constant domain (CH_1) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxyl terminus of the heavy chain CH_l domain including one or more cysteines from the antibody hinge region. F(ab') fragments are produced by cleavage of the disulfide bond at the hinge cysteines of the $F(ab')_2$ pepsin digestion product. Additional chemical couplings of antibody fragments are known to those of ordinary skill in the art.

[0064] In certain embodiments, the anti-VEGF antibodies of the disclosure are monoclonal antibodies. The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced. Monoclonal antibodies useful in connection with the present disclosure can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. The anti-VEGF antibodies of the disclosure include chimeric, primatized, humanized, or human antibodies.

[0065] The anti-VEGF antibodies of the disclosure can be chimeric antibodies. The term "chimeric" antibody as used herein refers to an antibody having variable sequences derived from a non-human immunoglobulin, such as rat or mouse antibody, and human immunoglobulin constant regions, typically chosen from a human immunoglobulin template. Methods for producing chimeric antibodies are known in the art. See, *e.g.*, Morrison, 1985, Science 229(4719):1202-7; Oi *et al.,* 1986, BioTechniques 4:214-221; Gillies *et al.,* 1985, J. Immunol. Methods 125:191-202; U.S. Pat. Nos. 5,807,715; 4,816,567; and 4,816397, which are incorporated herein by reference in their entireties.

[0066] The anti-VEGF antibodies of the disclosure can be humanized. "Humanized" forms of non-human (*e.g.*, murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', $F(ab')_2$ or other target-binding subdomains of antibodies) which contain minimal sequences derived from non-human immunoglobulin. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin sequence. The humanized antibody can also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin consensus sequence. Methods of antibody humanization are known in the art. See, *e.g.*, Riechmann *et al.,* 1988, Nature 332:323-7; U.S. Patent Nos: 5,530,101; 5,585,089; 5,693,761; 5,693,762; and 6,180,370 to Queen *et al.*; EP239400; PCT publication WO 91/09967; U.S. Patent No. 5,225,539; EP592106; EP519596; Padlan, 1991, Mol. Immunol., 28:489-498; Studnicka *et al.,* 1994, Prot. Eng. 7:805-814; Roguska *et al.,* 1994, Proc. Natl. Acad. Sci. 91:969-973; and U.S. Patent No. 5,565,332, all of which are hereby incorporated by reference in their entireties.

[0067] The anti-VEGF antibodies of the disclosure can be human antibodies. Completely "human" anti-VEGF antibodies can be desirable for therapeutic treatment of human patients. As used herein, “human antibodies” include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulin and that do not express endogenous immunoglobulins. Human antibodies can be made by a variety of methods known in the art including phage display methods using antibody libraries derived from human immunoglobulin sequences. See U.S. Patent Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645; WO 98/50433; WO 98/24893; WO 98/16654; WO 96/34096; WO 96/33735; and WO 91/10741, each of which is incorporated herein by reference in its entirety. Human antibodies can also be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. See, *e.g.*, PCT publications WO 98/24893; WO 92/01047; WO 96/34096; WO 96/33735; U.S. Patent Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806; 5,814,318; 5,885,793; 5,916,771; and 5,939,598, which are incorporated by reference herein in their entireties. In addition, companies such as Medarex (Princeton, NJ), Astellas Pharma (Deerfield, IL), Amgen (Thousand Oaks, CA) and Regeneron (Tarrytown, NY) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above. Completely human antibodies that recognize a selected epitope can be generated using a technique referred to as “guided selection.” In this approach a selected non-human monoclonal antibody, *e.g.*, a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope (Jespers *et al.,* 1988, Biotechnology 12:899-903).

[0068] The anti-VEGF antibodies of the disclosure can be primatized. The term “primatized antibody” refers to an antibody comprising monkey variable regions and human constant regions. Methods for producing primatized antibodies are known in the art. See *e.g.*, U.S. Patent Nos. 5,658,570; 5,681,722; and 5,693,780, which are incorporated herein by reference in their entireties.

[0069] The anti-VEGF antibodies of the disclosure can be bispecific antibodies. Bispecific antibodies are monoclonal, often human or humanized, antibodies that have binding specificities for at least two different antigens. In the present disclosure, one of the binding specificities can be directed towards VEGF, the other can be for any other antigen, *e.g.*, for a cell-surface protein, receptor, receptor subunit, tissue-specific antigen, virally derived protein, virally encoded envelope protein, bacterially derived protein, or bacterial surface protein, *etc.* In a specific embodiment, an antibody of the disclosure is a bispecific antibody with binding specificites for both VEGF and CD3.

[0070] The anti-VEGF antibodies of the disclosure include derivatized antibodies. For example, but not by way of limitation, derivatized antibodies are typically modified by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein (see Section 6.6 for a discussion of antibody conjugates), *etc.* Any of numerous chemical modifications can be carried out by known techniques, including, but not limited to, specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, *etc.* Additionally, the derivative can contain one or more non-natural amino acids, *e.g.*, using ambrx technology (see, *e.g.*, Wolfson, 2006, Chem. Biol. 13(10):1011-2).

[0071] In yet another embodiment of the disclosure, the anti-VEGF antibodies or fragments thereof can be antibodies or antibody fragments whose sequence has been modified to alter at least one constant region-mediated biological effector function relative to the corresponding wild type sequence.

[0072] For example, in some embodiments, an anti-VEGF antibody of the disclosure can be modified to reduce at least one constant region-mediated biological effector function relative to an unmodified antibody, *e.g.*, reduced binding to the Fc receptor (FcγR). FcγR binding can be reduced by mutating the immunoglobulin constant region segment of the antibody at particular regions necessary for FcγR interactions (see *e.g.*, Canfield and Morrison, 1991, J. Exp. Med. 173:1483-1491; and Lund *et al.,* 1991, J. Immunol. 147:2657-2662). Reduction in FcγR binding ability of the antibody can also reduce other effector functions which rely on FcγR interactions, such as opsonization, phagocytosis and antigen-dependent cellular cytotoxicity ("ADCC").

[0073] In other embodiments, an anti-VEGF antibody of the disclosure can be modified to acquire or improve at least one constant region-mediated biological effector function relative to an unmodified antibody, *e.g.*, to enhance FcγR interactions (*see, e.g.*, US 2006/0134709). For example, an anti-VEGF antibody of the disclosure can have a constant region that binds FcγRIIA, FcγRIIB and/or FcγRIIIA with greater affinity than the corresponding wild type constant region.

[0074] Thus, antibodies of the disclosure can have alterations in biological activity that result in increased or decreased opsonization, phagocytosis, or ADCC. Such alterations are known in the art. For example, modifications in antibodies that reduce ADCC activity are described in U.S. Patent No. 5,834,597. An exemplary ADCC lowering variant corresponds to “mutant 3” shown in Figure 4 of U.S. Patent No. 5,834,597, in which residue 236 is deleted and residues 234, 235 and 237 (using EU numbering) are substituted with alanines.

[0075] In some embodiments, the anti-VEGF antibodies of the disclosure have low levels of or lack fucose. Antibodies lacking fucose have been correlated with enhanced ADCC (activity, especially at low doses of antibody. See Shields *et al.*, 2002, J. Biol. Chem. 277:26733-26740; Shinkawa *et al.*, 2003, J. Biol. Chem. 278:3466-73. Methods of preparing fucose-less antibodies include growth in rat myeloma YB2/0 cells (ATCC CRL 1662). YB2/0 cells express low levels of FUT8 mRNA, which encodes α-1,6-fucosyltransferase, an enzyme necessary for fucosylation of polypeptides.

[0076] In yet another aspect, the anti-VEGF antibodies or fragments thereof can be antibodies or antibody fragments that have been modified to increase or reduce their binding affinities to the fetal Fc receptor, FcRn, for example by mutating the immunoglobulin constant region segment at particular regions involved in FcRn interactions (*see, e.g.*, WO 2005/123780). In particular embodiments, an anti-VEGF antibody of the IgG class is mutated such that at least one of amino acid residues 250, 314, and 428 of the heavy chain constant region is substituted alone, or in any combinations thereof, such as at positions 250 and 428, or at positions 250 and 314, or at positions 314 and 428, or at positions 250, 314, and 428, with positions 250 and 428 a specific combination. For position 250, the substituting amino acid residue can be any amino acid residue other than threonine, including, but not limited to, alanine, cysteine, aspartic acid, glutamic acid, phenylalanine, glycine, histidine, isoleucine, lysine, leucine, methionine, asparagine, proline, glutamine, arginine, serine, valine, tryptophan, or tyrosine. For position 314, the substituting amino acid residue can be any amino acid residue other than leucine, including, but not limited to, alanine, cysteine, aspartic acid, glutamic acid, phenylalanine, glycine, histidine, isoleucine, lysine, methionine, asparagine, proline, glutamine, arginine, serine, threonine, valine, tryptophan, or tyrosine. For position 428, the substituting amino acid residues can be any amino acid residue other than methionine, including, but not limited to, alanine, cysteine, aspartic acid, glutamic acid, phenylalanine, glycine, histidine, isoleucine, lysine, leucine, asparagine, proline, glutamine, arginine, serine, threonine, valine, tryptophan, or tyrosine. Specific combinations of suitable amino acid substitutions are identified in Table 1 of U.S. Patent No. 7,217,797, which table is incorporated by reference herein in its entirety. Such mutations increase the antibody's binding to FcRn, which protects the antibody from degradation and increases its half-life.

[0077] In yet other aspects, an anti-VEGF antibody has one or more amino acids inserted into one or more of its hypervariable regions, for example as described in Jung and Plückthun, 1997, Protein Engineering 10(9):959–966; Yazaki *et al.*, 2004, Protein Eng Des. Sel. 17(5):481–9. Epub 2004 Aug 17; and US 2007/0280931.

[0078] In various embodiments, the anti-VEGF antibodies or fragments thereof can be antibodies or antibody fragments that have been modified for increased expression in heterologous hosts. In certain embodiments, the anti-VEGF antibodies or fragments thereof can be antibodies or antibody fragments that have been modified for increased expression in and/or secretion from heterologous host cells. In some embodiments, the anti-VEGF antibodies or fragments thereof are modified for increased expression in bacteria, such as *E. coli*. In other embodiments, the anti-VEGF antibodies or fragments thereof are modified for increased expression in yeast (Kieke *et al.*, 1999, Proc. Nat'l Acad. Sci. USA 96:5651-5656). In still other embodiments, the anti-VEGF antibodies or fragments thereof are modified for increased expression in insect cells. In additional embodiments, the anti-VEGF antibodies or fragments thereof are modified for increased expression in mammalian cells, such as CHO cells.

[0079] In certain embodiments, the anti-VEGF antibodies or fragments thereof can be antibodies or antibody fragments that have been modified to increase stability of the antibodies during production. In some embodiments, the antibodies or fragments thereof can be modified to replace one or more amino acids such as asparagine or glutamine that are susceptible to nonenzymatic deamidation with amino acids that do not undergo deamidation (Huang *et al.*, 2005, Anal. Chem. 77:1432-1439). In other embodiments, the antibodies or fragments thereof can be modified to replace one or more amino acids that is susceptible to oxidation, such as methionine, cysteine or tryptophan, with an amino acid that does not readily undergo oxidation. In still other embodiments, the antibodies or fragments thereof can be modified to replace one or more amino acids that is susceptible to cyclization, such as asparagine or glutamic acid, with an amino acid that does not readily undergo cyclization.

6.2 NUCLEIC ACIDS AND EXPRESSION SYSTEMS

[0080] The present disclosure encompasses nucleic acid molecules and host cells encoding the anti-VEGF antibodies of the disclosure.

[0081] An anti-VEGF antibody of the disclosure can be prepared by recombinant expression of immunoglobulin light and heavy chain genes in a host cell. To express an antibody recombinantly, a host cell is transfected with one or more recombinant expression vectors carrying DNA fragments encoding the immunoglobulin light and heavy chains of the antibody such that the light and heavy chains are expressed in the host cell and, optionally, secreted into the medium in which the host cells are cultured, from which medium the antibodies can be recovered. Standard recombinant DNA methodologies are used to obtain antibody heavy and light chain genes, incorporate these genes into recombinant expression vectors and introduce the vectors into host cells, such as those described in Molecular Cloning; A Laboratory Manual, Second Edition (Sambrook, Fritsch and Maniatis (eds), Cold Spring Harbor, N. Y., 1989), Current Protocols in Molecular Biology (Ausubel, F.M. *et al.,* eds., Greene Publishing Associates, 1989) and in U.S. Patent No. 4,816,397.

[0082] In one embodiment, the anti-VEGF antibodies are similar to bevacizumab or ranibizumab but for changes in one or more CDRs. In another embodiment, the anti-VEGF antibodies are similar to bevacizumab or ranibizumab but for changes in one or more framework regions. In yet another embodiment, the anti-VEGF antibodies are similar to bevacizumab or ranibizumab but for changes in one or more CDRs and in one or more framework regions. Such antibodies are referred to herein collectively as having "bevacizumab-related" or "ranibizumab-related" sequences and are sometimes referenced simply as anti-VEGF antibodies of the disclosure. To generate nucleic acids encoding anti-VEGF antibodies, DNA fragments encoding the light and heavy chain variable regions are first obtained. These DNAs can be obtained by amplification and modification of germline DNA or cDNA encoding light and heavy chain variable sequences, for example using the polymerase chain reaction (PCR). Germline DNA sequences for human heavy and light chain variable region genes are known in the art (*see, e.g.*, the "VBASE" human germline sequence database; see also Kabat, E. A. *et al.,* 1991, Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242; Tomlinson *et al.,* 1992, J. Mol. Biol. 22T:116-198; and Cox *et al.,* 1994, Eur. J. Immunol. 24:827-836; the contents of each of which are incorporated herein by reference). A DNA fragment encoding the heavy or light chain variable region of bevacizumab or ranibizumab can be synthesized and used as a template for mutagenesis to generate a variant as described herein using routine mutagenesis techniques; alternatively, a DNA fragment encoding the variant can be directly synthesized.

[0083] Once DNA fragments encoding anti-VEGF VH and VL segments are obtained, these DNA fragments can be further manipulated by standard recombinant DNA techniques, for example to convert the variable region genes to full-length antibody chain genes, to Fab fragment genes or to a scFv gene. In these manipulations, a VL- or VH-encoding DNA fragment is operatively linked to another DNA fragment encoding another protein, such as an antibody constant region or a flexible linker. The term "operatively linked," as used in this context, is intended to mean that the two DNA fragments are joined such that the amino acid sequences encoded by the two DNA fragments remain in-frame.

[0084] The isolated DNA encoding the VH region can be converted to a full-length heavy chain gene by operatively linking the VH-encoding DNA to another DNA molecule encoding heavy chain constant regions (CH_1, CH_2, CH_3 and, optionally, CH_4). The sequences of human heavy chain constant region genes are known in the art (*see, e.g.*, Kabat, E.A. *et al.,* 1991, Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242) and DNA fragments encompassing these regions can be obtained by standard PCR amplification. The heavy chain constant region can be an IgG_1, IgG_2, IgG_3, IgG_4, IgA, IgE, IgM or IgD constant region, but in certain embodiments is an IgG_1 or IgG_4 constant region. For a Fab fragment heavy chain gene, the VH-encoding DNA can be operatively linked to another DNA molecule encoding only the heavy chain CH_1 constant region.

[0085] The isolated DNA encoding the VL region can be converted to a full-length light chain gene (as well as a Fab light chain gene) by operatively linking the VL-encoding DNA to another DNA molecule encoding the light chain constant region, CL. The sequences of human light chain constant region genes are known in the art (*see, e.g.*, Kabat, E. A. *et al.,* 1991, Sequences of Proteins of Immunological Interest, Fifth Edition (U.S. Department of Health and Human Services, NIH Publication No. 91-3242)) and DNA fragments encompassing these regions can be obtained by standard PCR amplification. The light chain constant region can be a kappa or lambda constant region, but in certain embodiments is a kappa constant region. To create a scFv gene, the VH- and VL-encoding DNA fragments are operatively linked to another fragment encoding a flexible linker, *e.g.*, encoding the amino acid sequence $(Gly_4{\sim}Ser)_3$, such that the VH and VL sequences can be expressed as a contiguous single-chain protein, with the VL and VH regions joined by the flexible linker (*see, e.g.*, Bird *et al.,* 1988, Science 242:423-426; Huston *et al.,* 1988, Proc. Natl. Acad. Sci. USA 85:5879-5883; McCafferty *et al.*, 1990, Nature 348:552-554).

[0086] To express the anti-VEGF antibodies of the disclosure, DNAs encoding partial or full-length light and heavy chains, obtained as described above, are inserted into expression vectors such that the genes are operatively linked to transcriptional and translational control sequences. In this context, the term “operatively linked” is intended to mean that an antibody gene is ligated into a vector such that transcriptional and translational control sequences within the vector serve their intended function of regulating the transcription and translation of the antibody gene. The expression vector and expression control sequences are chosen to be compatible with the expression host cell used. The antibody light chain gene and the antibody heavy chain gene can be inserted into separate vectors or, more typically, both genes are inserted into the same expression vector.

[0087] The antibody genes are inserted into the expression vector by standard methods (*e.g.*, ligation of complementary restriction sites on the antibody gene fragment and vector, or blunt end ligation if no restriction sites are present). Prior to insertion of the light or heavy chain sequences of the anti-VEGF antibodies of the disclosure, the expression vector can already carry antibody constant region sequences. For example, one approach to converting the anti-VEGF VH and VL sequences to full-length antibody genes is to insert them into expression vectors already encoding heavy chain constant and light chain constant regions, respectively, such that the VH segment is operatively linked to the CH segment(s) within the vector and the VL segment is operatively linked to the CL segment within the vector. Additionally or alternatively, the recombinant expression vector can encode a signal peptide that facilitates secretion of the antibody chain from a host cell. The antibody chain gene can be cloned into the vector such that the signal peptide is linked in-frame to the amino terminus of the antibody chain gene. The signal peptide can be an immunoglobulin signal peptide or a heterologous signal peptide (*i.e.*, a signal peptide from a non-immunoglobulin protein).

[0088] In addition to the antibody chain genes, the recombinant expression vectors of the disclosure carry regulatory sequences that control the expression of the antibody chain genes in a host cell. The term “regulatory sequence” is intended to include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals) that control the transcription or translation of the antibody chain genes. Such regulatory sequences are described, for example, in Goeddel, Gene Expression Technology: Methods in Enzymology 185 (Academic Press, San Diego, CA, 1990). It will be appreciated by those skilled in the art that the design of the expression vector, including the selection of regulatory sequences may depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, *etc.* Suitable regulatory sequences for mammalian host cell expression include viral elements that direct high levels of protein expression in mammalian cells, such as promoters and/or enhancers derived from cytomegalovirus (CMV) (such as the CMV promoter/enhancer), Simian Virus 40 (SV40) (such as the SV40 promoter/enhancer), adenovirus, (*e.g.*, the adenovirus major late promoter (AdMLP)) and polyoma. For further description of viral regulatory elements, and sequences thereof, see *e.g.*, U.S. Patent No. 5,168,062 by Stinski, U.S. Patent No. 4,510,245 by Bell *et al.,* and U.S. Patent No. 4,968,615 by Schaffner *et al.,*

[0089] In addition to the antibody chain genes and regulatory sequences, the recombinant expression vectors of the disclosure can carry additional sequences, such as sequences that regulate replication of the vector in host cells (*e.g.*, origins of replication) and selectable marker genes. The selectable marker gene facilitates selection of host cells into which the vector has been introduced (see *e.g.*, U.S. Patents Nos. 4,399,216, 4,634,665 and 5,179,017, all by Axel *et al.*). For example, typically the selectable marker gene confers resistance to drugs, such as G418, puromycin, blasticidin, hygromycin or methotrexate, on a host cell into which the vector has been introduced. Suitable selectable marker genes include the dihydrofolate reductase (DHFR) gene (for use in $DHFR^-$ host cells with methotrexate selection/amplification) and the neo gene (for G418 selection). For expression of the light and heavy chains, the expression vector(s) encoding the heavy and light chains is transfected into a host cell by standard techniques. The various forms of the term "transfection" are intended to encompass a wide variety of techniques commonly used for the introduction of exogenous DNA into a prokaryotic or eukaryotic host cell, *e.g.*, electroporation, lipofection, calcium-phosphate precipitation, DEAE- dextran transfection and the like.

[0090] It is possible to express the antibodies of the disclosure in either prokaryotic or eukaryotic host cells. In certain embodiments, expression of antibodies is performed in eukaryotic cells, *e.g.*, mammalian host cells, for optimal secretion of a properly folded and immunologically active antibody. Exemplary mammalian host cells for expressing the recombinant antibodies of the disclosure include Chinese Hamster Ovary (CHO cells) (including $DHFR^-$ CHO cells, described in Urlaub and Chasin, 1980, Proc. Natl. Acad. Sci. USA 77:4216-4220, used with a DHFR selectable marker, *e.g.*, as described in Kaufman and Sharp, 1982, Mol. Biol. 159:601-621), NS0 myeloma cells, COS cells, 293 cells and SP2/0 cells. When recombinant expression vectors encoding antibody genes are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or secretion of the antibody into the culture medium in which the host cells are grown. Antibodies can be recovered from the culture medium using standard protein purification methods. Host cells can also be used to produce portions of intact antibodies, such as Fab fragments or scFv molecules. It is understood that variations on the above procedure are within the scope of the present disclosure. For example, it can be desirable to transfect a host cell with DNA encoding either the light chain or the heavy chain (but not both) of an anti-VEGF antibody of this disclosure.

[0091] Recombinant DNA technology can also be used to remove some or all of the DNA encoding either or both of the light and heavy chains that is not necessary for binding to VEGF. The molecules expressed from such truncated DNA molecules are also encompassed by the antibodies of the disclosure.

[0092] In addition, bifunctional antibodies can be produced in which one heavy and one light chain are an antibody of the disclosure and the other heavy and light chain are specific for an antigen other than VEGF by crosslinking an antibody of the disclosure to a second antibody by standard chemical crosslinking methods. Bifunctional antibodies can also be made by expressing a nucleic acid engineered to encode a bifunctional antibody.

[0093] In certain embodiments, dual specific antibodies, *i.e.,* antibodies that bind VEGF and an unrelated antigen using the same binding site, can be produced by mutating amino acid residues in the light chain and/or heavy chain CDRs. In various embodiments, dual specific antibodies that bind two antigens, such as HER2 and VEGF, can be produced by mutating amino acid residues in the periphery of the antigen binding site (Bostrom *et al.,* 2009, Science 323:1610-1614). Dual functional antibodies can be made by expressing a nucleic acid engineered to encode a dual specific antibody.

[0094] For recombinant expression of an anti-VEGF antibody of the disclosure, the host cell can be co-transfected with two expression vectors of the disclosure, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. Typically, the two vectors each contain a separate selectable marker. Alternatively, a single vector can be used which encodes both heavy and light chain polypeptides.

[0095] Once a nucleic acid encoding one or more portions of an anti-VEGF antibody is generated, further alterations or mutations can be introduced into the coding sequence, for example to generate nucleic acids encoding antibodies with different CDR sequences, antibodies with reduced affinity to the Fc receptor, or antibodies of different subclasses.

[0096] The anti-VEGF antibodies of the disclosure can also be produced by chemical synthesis (*e.g.*, by the methods described in Solid Phase Peptide Synthesis, 2^{nd} ed., 1984

The Pierce Chemical Co., Rockford, Ill.). Variant antibodies can also be generated using a cell-free platform (*see, e.g.*, Chu *et al.*, 2001, Biochemia No. 2 (Roche Molecular Biologicals)).

[0097] Once an anti-VEGF antibody of the disclosure has been produced by recombinant expression, it can be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (*e.g.*, ion exchange, affinity, particularly by affinity for VEGF after Protein A or Protein G selection, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. Further, the anti-VEGF antibodies of the present disclosure or fragments thereof can be fused to heterologous polypeptide sequences described herein or otherwise known in the art to facilitate purification.

[0098] Once isolated, an anti-VEGF antibody can, if desired, be further purified, *e.g.*, by high performance liquid chromatography (See, *e.g.*, Fisher, Laboratory Techniques In Biochemistry And Molecular Biology (Work and Burdon, eds., Elsevier, 1980), or by gel filtration chromatography on a Superdex^{TM} 75 column (Pharmacia Biotech AB, Uppsala, Sweden).

6.3 BIOLOGICAL ACTIVITIES OF ANTI-VEGF ANTIBODIES

[0099] In certain embodiments, the anti-VEGF antibodies of the disclosure have certain biological activities, such as competing with bevacizumab or ranibizumab for binding to VEGF or neutralizing VEGF activity.

[0100] Accordingly, in certain embodiments, anti-VEGF antibodies of the disclosure compete with bevacizumab or ranibizumab for binding to VEGF. The ability to compete for binding to VEGF can be tested using a competition assay. In one example of a competition assay, VEGF is adhered onto a solid surface, *e.g.*, a microwell plate, by contacting the plate with a solution of VEGF (*e.g.*, at a concentration of 1 μg/mL in PBS over night at 4°C). The plate is washed (*e.g.*, 0.1% Tween 20 in PBS) and blocked (*e.g.*, in Superblock, Thermo Scientific, Rockford, IL). A mixture of sub-saturating amount of biotinylated bevacizumab (80 ng/mL) or an equivalent amount of biotinylated ranibizumab and unlabeled bevacizumab (or ranibizumab as the case may be) (the "reference" antibody) or competing anti-VEGF antibody (the "test" antibody) antibody in serial dilution (*e.g.*, at a concentration of 2.8 μg/mL, 8.3 μg/mL, or 25 μg/mL) in ELISA buffer (*e.g.*, 1% BSA and 0.1% Tween 20 in PBS) is added to wells and plates are incubated for 1 hour with gentle shaking. The plate is washed, 1μg/mL HRP-conjugated Streptavidin diluted in ELISA buffer is added to each well and the plates incubated for 1 hour. Plates are washed and bound antibodies were detected by addition of substrate (*e.g.*, TMB, Biofx Laboratories Inc., Owings Mills, MD). The reaction is terminated by addition of stop buffer (*e.g.*, Bio FX Stop Reagents, Biofx Laboratories Inc., Owings Mills, MD) and the absorbance is measured at 650 nm using microplate reader (*e.g.*, VERSAmax, Molecular Devices, Sunnyvale, CA). Variations on this competition assay can also be used to test competition between an anti-VEGF antibody of the disclosure and bevacizumab or ranibizumab. For example, in certain aspects, the anti-VEGF antibody is used as a reference antibody and bevacizumab or ranibizumab is used as a test antibody. Additionally, instead of soluble VEGF, membrane-bound VEGF expressed on the surfaces of cell (for example mammalian cells) in culture can be used. Other formats for competition assays are known in the art and can be employed.

[0101] In various embodiments, an anti-VEGF antibody of the disclosure reduces the binding of labeled bevacizumab or ranibizumab by at least 30%, by at least 40%, by at least 50%, by at least 60%, by at least 70%, by at least 80%, by at least 90%, by at least 95%, by at least 99% or by a percentage ranging between any of the foregoing values (*e.g.*, an anti-VEGF antibody of the disclosure reduces the binding of labeled bevacizumab or ranibizumab by 50% to 70%) when the anti-VEGF antibody is used at a concentration of 0.08 μg/mL, 0.4 μg/mL, 2 μg/mL, 10 μg/mL, 50 μg/mL, 100 μg/mL or at a concentration ranging between any of the foregoing values (*e.g.*, at a concentration ranging from 2 μg/mL to 10 μg/mL).

[0102] In other embodiments, bevacizumab or ranibizumab reduces the binding of a labeled anti-VEGF antibody of the disclosure by at least 40%, by at least 50%, by at least 60%, by at least 70%, by at least 80%, by at least 90%, or by a percentage ranging between any of the foregoing values (*e.g.*, bevacizumab or ranibizumab reduces the binding of a labeled an anti-VEGF antibody of the disclosure by 50% to 70%) when bevacizumab or ranibizumab is used at a concentration of 0.4 μg/mL, 2 μg/mL, 10 μg/mL, 50 μg/mL, 250 μg/mL or at a concentration ranging between any of the foregoing values (*e.g.*, at a concentration ranging from 2 μg/mL to 10 μg/mL).

[0103] In other aspects, an anti-VEGF antibody of the disclosure inhibits (or neutralizes) VEGF activity in a range of *in vitro* assays, such as cell proliferation or cell migration. For example, in one embodiment, the VEGF activity assayed is induction of endothelial cell ("EC") proliferation (see, *e.g.*, protocol of Qin *et al.*, 2006, J. Biol. Chem. 281:32550-32558). In another embodiment, the VEGF activity assayed is induction of EC migration (see, *e.g.*, the *in vitro* scratch assay protocol described of Liang *et al.*, 2007, Nat. Protoc. 2:329-333). In a specific embodiment, an anti-VEGF antibody is tested for the ability to reverse proliferation and cell migration stimulated by VEGF and delocalization of tight junction proteins induced by $VEGF_{165}$ in immortalized bovine retinal endothelial cells (Deissler *et al.*, 2008, British Journal of Ophthalmology 92:839-843). In yet another embodiment, the neutralization of VEGF activity is assayed using a reporter assay (see, *e.g.*, Yohno *et al.*, 2003, Biological & Pharmaceutical Bulletin 26(4):417-20 and U.S. Patent No. 6,787,323).

[0104] Other formats for VEGF neutralization assays are known in the art and can be employed.

[0105] In various embodiments, an anti-VEGF antibody of the disclosure neutralizes VEGF by at least 30%, by at least 40%, by at least 50%, by at least 60%, by at least 70%, by at least 80%, by at least 90%, or by a percentage ranging between any of the foregoing values (*e.g.*, an anti-VEGF antibody of the disclosure neutralizes VEGF activity by 50% to 70%) when the anti-VEGF antibody is used at a concentration of 2 ng/mL, 5 ng/mL, 10 ng/mL, 20 ng/mL, 0.1 μg/mL, 0.2 μg/mL, 1 μg/mL, 2 μg/mL, 5 μg/mL, 10 μg/mL, 20 μg/mL, or at a concentration ranging between any of the foregoing values (*e.g.*, at a concentration ranging from 1 μg/mL to 5 μg/mL).

[0106] In some embodiments, an anti-VEGF antibody of the disclosure is at least 0.7-fold as effective, 0.8-fold as effective, at least 0.9-fold as effective, at least 1-fold as effective, at least 1.1-fold as effective, at least 1.25-fold as effective, at least 1.5-fold as effective, at least 2-fold as effective, at least 5-fold as effective, at least 10-fold as effective, at least 20-fold as effective, at least 50-fold as effective, at least 100-fold as effective, at least 200-fold as effective, at least 500-fold as effective, at least 1000-fold as effective as bevacizumab or ranibizumab at neutralizing VEGF, or having an effectiveness at neutralizing VEGF relative to bevacizumab or ranibizumab ranging between any pair of the foregoing values (*e.g.*, 0.9-fold to 5-fold as effective as bevacizumab or ranibizumab or 2-fold to 50-fold as effective as bevacizumab or ranibizumab in neutralizing VEGF).

6.4 KINETIC PROPERTIES OF ANTI-VEGF ANTIBODIES

[0107] In certain embodiments, the anti-VEGF antibodies of the disclosure have a high binding affinity for VEGF. In specific embodiments, the anti-VEGF antibodies of the present disclosure have specific association rate constants (k_{on} or k_a values), dissociation rate constants (k_{off} or k_d values), affinity constants (K_A values), dissociation constants (K_D values) and/or IC_{50} values. In various embodiments, binding constants for the interaction of the anti-VEGF antibodies with VEGF receptor can be determined using surface plasmon resonance, *e.g.,* according to the method disclosed in Karlsson *et al.,* 1991, J. Immunol. Methods 145:229-240. In certain aspects, such values are selected from the following embodiments.

[0108] In a specific embodiment, an anti-VEGF antibody of the disclosure binds to VEGF with a k_{on} of at least 10^4 $M^{-1}s^{-1}$, at least 5 X 10^4 $M^{-1}s^{-1}$, at least 10^5 $M^{-1}s^{-1}$, at least 5 X 10^5 $M^{-1}s^{-1}$, at least 10^6 $M^{-1}s^{-1}$, at least 5 X 10^6 $M^{-1}s^{-1}$, at least 10^7 $M^{-1}s^{-1}$, at least 5 X 10^7 $M^{-1}s^{-1}$, at least 10^8 $M^{-1}s^{-1}$, at least 5 X 10^8 $M^{-1}s^{-1}$, at least 10^9 $M^{-1}s^{-1}$or with a k_{on} of any range between any pair of the foregoing values (*e.g.*, 5 X 10^5 to 5 X 10^6 $M^{-1}s^{-1}$ or 10^7 to 10^8 $M^{-1}s^{-1}$).

[0109] In another embodiment, an anti-VEGF antibody of the disclosure binds to VEGF with a k_{off} rate of 10^{-3} s^{-1} or less, 5 X 10^{-4} s^{-1}or less, 10^{-4} s^{-1} or less, 5 X 10^{-5} s^{-1} or less, 10^{-5} s^{-1} or less, 5 X 10^{-6} s^{-1} or less, 10^{-6} s^{-1} or less, 5 X 10^{-7} s^{-1} or less, 10^{-7} s^{-1} or less, 5 X 10^{-8} s^{-1} or less, 10^{-8} s^{-1} or less, or with a k_{off} rate of any range between any pair of the foregoing values (*e.g.*, 5 X 10^{-4} to 10^{-6} s^{-1}, or 10^{-3} to 5 X 10^{-5} s^{-1}).

[0110] In another embodiment, an anti-VEGF antibody of the disclosure binds to VEGF with a K_A (k_{on}/k_{off}) of at least at least 10^8 M^{-1}, at least 5 X 10^9 M^{-1}, at least 10^{10} M^{-1}, at least 5 X 10^{10} M^{-1}, 10^{11} M^{-1}, at least 5 X 10^{11} M^{-1}, at least 10^{12} M^{-1}, at least 5 X 10^{12} M^{-1}, at least 10^{13} M^{-1}, at least 5 X 10^{13} M^{-1}, at least 10^{14} M^{-1}, at least 5 X 10^{14} M^{-1}, at least 10^{15} M^{-1}or with a K_A of any range between any pair of the foregoing values (*e.g.*, from 5 X 10^9 M^{-1} to 10^{11} M^{-1}, or from 10^{11} M^{-1} to 5 X 10^{14} M^{-1}).

[0111] In other embodiments, an anti-VEGF antibody of the disclosure binds to VEGF with a K_D (k_{off}/k_{on}) of 10^{-8} M or less, 5 X 10^{-9} M or less, 10^{-9} M or less, 5 X 10^{-10} M or less, 10^{-10} M or less, 5 X 10^{-11} M or less, 10^{-11} M or less, 5 X 10^{-12} M or less, 10^{-12} M or less, 5 X 10^{-13} M or less, 10^{-13} M or less, 5 X 10^{-14} M or less, 10^{-14} M or less, 5 X 10^{-15} M or less, 10^{-15} M or less, or with a K_D of any range between any pair of the foregoing values (*e.g.*, 5 X 10^{-9} to5 X 10^{-12} M, or from 5 X $10^{-11\,M}$ to 5 X 10^{-13} M).

[0112] In specific embodiments, the K_D (k_{off}/k_{on}) value is determined by assays well known in the art or described herein, *e.g.*, ELISA, isothermal titration calorimetry (ITC), fluorescent polarization assay or any other biosensors such as BIAcore.

[0113] In some embodiments, an anti-VEGF antibody of the disclosure binds to VEGF and inhibits the binding of VEGF to a VEGF receptor (Flt-1 or Flk-1) at an IC_{50} value of less than 5 X 10^7 nM, less than 10^7 nM, less than 5 X 10^6 nM, less than 10^6 nM, less than 5 X 10^5 nM, less than 10^5 nM, less than 5 X 10^4 nM, less than 10^4 nM, less than 5 X 10^3 nM, less than 10^3 nM, less than 5 X 10^2 nM, less than 100 nM, less than 90 nM, less than 80 nM, less than 70 nM, less than 65 nM, less than 60 nM, less than 50 nM, less than 40 nM, less than 30 nM, less than 25 nM, less than 20 nM, less than 15 nM, less than 12 nM, less than 10 nM, less than 5 nM, less than 1 nM, less than 5 X 10^{-1} nM, less than 10^{-1} nM, less than 5 X 10^{-2} nM, less than 10^{-2} nM, less than 5 X 10^{-3} nM, less than 10^{-3} nM, less than 5 X 10^{-4} nM, or less than 10^{-4} nM, or with an IC_{50} of any range between any pair of the foregoing values (*e.g.*, 5 X 10^7 to 50 nM, or 15 nM to 5 X 10^{-3} nM). IC_{50} can be measured according to methods well known in the art or described herein, *e.g.*, ELISA.

[0114] In other embodiments, an anti-VEGF antibody of the disclosure binds to VEGF and neutralizes the activity VEGF in a bioassay (*e.g.*, EC proliferation or migration) at an IC_{50} value of less than 5 X 10^7 nM, less than 10^7 nM, less than 5 X 10^6 nM, less than 10^6 nM, less than 5 X 10^5 nM, less than 10^5 nM, less than 5 X 10^4 nM, less than 10^4 nM, less than 5 X 10^3 nM, less than 10^3 nM, less than 5 X 10^2 nM, less than 100 nM, less than 90 nM, less than 80 nM, less than 70 nM, less than 65 nM, less than 60 nM, less than 50 nM, less than 40 nM, less than 30 nM, less than 25 nM, less than 20 nM, less than 15 nM, less than 12 nM, less than 10 nM, less than 5 nM, less than 1 nM, less than 5 X 10^{-1} nM, less than 10^{-1} nM, less than 5 X 10^{-2} nM, less than 10^{-2} nM, less than 5 X 10^{-3} nM, less than 10^{-3} nM, less than 5 X 10^{-4} nM, or less than 10^{-4} nM, or with an IC_{50} of any range between any pair of the foregoing values (*e.g.*, 5 X 10^7 to 50 nM, or 15 nM to 5 X 10^{-3} nM). An exemplary neutralization assay that can be used to measure the IC_{50} of an anti-VEGF antibody is described in Section 6.3 below.

[0115] In certain embodiments, an anti-VEGF antibody binds to VEGF and inhibits the binding of VEGF to Flt-1, Flk-1 or both, or inhibits VEGF activity in a VEGF neutralization assay, at an IC_{50} value of between approximately 1 pm and approximately 1 μM. In specific embodiments, an anti-VEGF antibody binds to VEGF and inhibits the binding of VEGF to Flt-1, Flk-1 or both, or inhibits VEGF activity in a VEGF neutralization assay, at an IC_{50} value of between 10 pM and 100 nM, between 100 pM and 10 nM, between 200 pM and 5 nM, between 300 pM and 4 nM, between 500 pM and 3 nM, between 750 pM and 2 nM, between 1 nM and 20 nM, between 500 pM and 40 nM, between 50 pM and 50 nM, between 250 pM and 100 nM, and between 100 nM and 1 μM, or with an IC_{50} of any range between any pair of the foregoing values (*e.g.*, 10 pM to 50 nM, or 750 pM to 2 nM).

[0116] In certain aspects of the foregoing embodiments, the IC_{50} is measured in the presence of VEGF at a concentration of 0.001 μM, 0.005 μM, 0.01 μM, 0.05 μM, 0.1 μM, 0.5 μM, 1 μM, 10 μM, 20 μM, 30 μM, 40 μM, 50 μM, 60 μM, 70 μM, 80 μM, 90 μM, 100 μM, 200 μM, 300 μM, 400 μM, 500 μM, 600 μM, 700 μM, 800 μM, 900 μM, 1000 μM or at a concentration of any range between any pair of the foregoing values (*e.g.*, 0.01 to 50 μM, or 10 μM to 100 μM).

[0117] In certain embodiments, the kinetic properties of an antibody of the disclosure are comparable to, or improved relative to, bevacizumab or of ranibizumab in a comparable assay. For example, in certain embodiments, an anti-VEGF antibody of the disclosure binds to VEGF with a k_{on} rate ranging from approximately 0.5x to 1000x of the k_{on} of bevacizumab or of ranibizumab, for example a k_{on} of 0.5x of the k_{on} of bevacizumab or of ranibizumab, a k_{on} of 0.75x of the k_{on} of bevacizumab or of ranibizumab, a k_{on} of 0.9x of the k_{on} of bevacizumab or of ranibizumab, a k_{on} of 1x of the k_{on} of bevacizumab or of ranibizumab, a k_{on} of 1.1x of the k_{on} of bevacizumab or of ranibizumab, a k_{on} of 1.2x of the k_{on} of bevacizumab or of ranibizumab, a k_{on} of 1.3x of the k_{on} of bevacizumab or of ranibizumab, a k_{on} of 1.4x of the k_{on} of bevacizumab or of ranibizumab, a k_{on} of 1.5x of the k_{on} of bevacizumab or of ranibizumab, a k_{on} of 1.75x of the k_{on} of bevacizumab or of ranibizumab, a k_{on} of 2x of the k_{on} of bevacizumab or of ranibizumab, a k_{on} of 2.25x of the k_{on} of bevacizumab or of ranibizumab, a k_{on} of 2.5x of the k_{on} of bevacizumab or of ranibizumab, a k_{on} of 3x of the k_{on} of bevacizumab or of ranibizumab, a k_{on} of 4x of the k_{on} of bevacizumab or of ranibizumab, a k_{on} of 5x of the k_{on} of bevacizumab or of ranibizumab, a k_{on} of 7.5x of the k_{on} of bevacizumab or of ranibizumab, a k_{on} of 10x of the k_{on} of bevacizumab or of ranibizumab, a k_{on} of 15x of the k_{on} of bevacizumab or of ranibizumab, a k_{on} of 20x of the k_{on} of bevacizumab or of ranibizumab, a k_{on} of 50x of the k_{on} of bevacizumab or of ranibizumab, a k_{on} of 75x of the k_{on} of bevacizumab or of ranibizumab, a k_{on} of 100x of the k_{on} of bevacizumab or of ranibizumab, a k_{on} of 150x of the k_{on} of bevacizumab or of ranibizumab, a k_{on} of 200x of the k_{on} of bevacizumab or of ranibizumab, a k_{on} of 200x of the k_{on} of bevacizumab or of ranibizumab or a k_{on} ranging between any pair of the foregoing values, *e.g.*, a k_{on} of 2x-75x of the k_{on} of bevacizumab or of ranibizumab, a k_{on} of 5x-100x of the k_{on} of bevacizumab or of ranibizumab, a k_{on} of 0.5x-1000x of the k_{on} of bevacizumab or of ranibizumab, a k_{on} of 0.75x-200x of the k_{on} of bevacizumab or of ranibizumab, etc.

[0118] In certain embodiments, an anti-VEGF antibody of the disclosure binds to VEGF with a k_{off} rate ranging from 0.001x to 3x of the k_{off} of bevacizumab or of ranibizumab, for example a k_{off} of 0.002x of the k_{off} of bevacizumab or of ranibizumab, a k_{off} of 0.005x of the k_{off} of bevacizumab or of ranibizumab, a k_{off} of 0.0075x of the k_{off} of bevacizumab or of ranibizumab, a k_{off} of 0.01x of the k_{off} of bevacizumab or of ranibizumab, a k_{off} of 0.025x of the k_{off} of bevacizumab or of ranibizumab, a k_{off} of 0.05x of the k_{off} of bevacizumab or of ranibizumab, a k_{off} of 0.075x of the k_{off} of bevacizumab or of ranibizumab, a k_{off} of 0.1x of the k_{off} of bevacizumab or of ranibizumab, a k_{off} of 0.25x of the k_{off} of bevacizumab or of ranibizumab, a k_{off} of 0.5x of the k_{off} of bevacizumab or of ranibizumab, a k_{off} of 0.75x of the k_{off} of bevacizumab or of ranibizumab, a k_{off} of 0.9x of the k_{off} of bevacizumab or of ranibizumab, a k_{off} of 1x of the k_{off} of bevacizumab or of ranibizumab, a k_{off} of 1.1x of the k_{off} of bevacizumab or of ranibizumab, a k_{off} of 1.25x of the k_{off} of bevacizumab or of ranibizumab, a k_{off} of 1.5x of the k_{off} of bevacizumab or of ranibizumab, a k_{off} of 1.75x of the k_{off} of bevacizumab or of ranibizumab, a k_{off} of 4x of the k_{off} of bevacizumab or of ranibizumab, a k_{off} of 3x of the k_{off} of bevacizumab or of ranibizumab, a k_{off} of 2x of the k_{off} of bevacizumab or of ranibizumab, a k_{off} of 3x of the k_{off} of bevacizumab or of ranibizumab, or a k_{off} ranging between any pair of the foregoing values, *e.g.*, a k_{off} of 0.01x to 1.1x of the k_{off} of bevacizumab or of ranibizumab, a k_{off} of 0.05x-1.25x of the k_{off} of bevacizumab or of ranibizumab, a k_{off} of 0.1x-0.9x of the k_{off} of bevacizumab or of ranibizumab, etc.

[0119] In other embodiments, an anti-VEGF antibody of the disclosure binds to VEGF with a K_A (k_{on}/k_{off}) ranging from 0.25x to 1000x of the K_A of bevacizumab or of ranibizumab, for example a K_A of 0.5x of the K_A of bevacizumab or of ranibizumab, a K_A of 0.75x of the K_A of bevacizumab or of ranibizumab, a K_A of 1x of the K_A of bevacizumab or of ranibizumab, a K_A of 2x of the K_A of bevacizumab or of ranibizumab, a K_A of 4x of the K_A of bevacizumab or of ranibizumab, a K_A of 10x of the K_A of bevacizumab or of ranibizumab, a K_A of 15x of the K_A of bevacizumab or of ranibizumab, a K_A of 20x of the K_A of bevacizumab or of ranibizumab, a K_A of 30x of the K_A of bevacizumab or of ranibizumab, a K_A of 40x of the K_A of bevacizumab or of ranibizumab, a K_A of 50x of the K_A of bevacizumab or of ranibizumab, a K_A of 100x of the K_A of bevacizumab or of ranibizumab, a K_A of 250x of the K_A of bevacizumab or of ranibizumab, a K_A of 500x of the K_A of bevacizumab or of ranibizumab, a K_A of 750x of the K_A of bevacizumab or of ranibizumab, a K_A of 1000x of the K_A of bevacizumab or of ranibizumab or a K_A ranging between any pair of the foregoing values, *e.g.*, a K_A of 0.75x-10 5x of the K_A of bevacizumab or of ranibizumab, a K_A of 1x-100x of the K_A of bevacizumab or of ranibizumab, a K_A of 10x-20x of the K_A of bevacizumab or of ranibizumab, a K_A of 4x-50x of the K_A of bevacizumab or of ranibizumab, a K_A of 2x-20x of the K_A of bevacizumab or of ranibizumab, or any value or range that can be calculated from the k_{on} and k_{off} rates disclosed herein.

[0120] In other embodiments, an anti-VEGF antibody of the disclosure binds to VEGF a K_D (k_{off}/k_{on}) ranging from ranging from 0.001 x to 10x of the K_D of bevacizumab or of ranibizumab, for example a K_D of 0.001x of the K_D of bevacizumab or of ranibizumab, a K_D of 0.005x of the K_D of bevacizumab or of ranibizumab, a K_D of 0.01x of the K_D of bevacizumab or of ranibizumab, a K_D of 0.05x of the K_D of bevacizumab or of ranibizumab, a K_D of 0.075x of the K_D of bevacizumab or of ranibizumab, a K_D of 0.1x of the K_D of bevacizumab or of ranibizumab, a K_D of 0.2x of the K_D of bevacizumab or of ranibizumab, a K_D of 0.3x of the K_D of bevacizumab or of ranibizumab, a K_D of 0.4x of the K_D of bevacizumab or of ranibizumab, a K_D of 0.5x of the K_D of bevacizumab or of ranibizumab, a K_D of 0.6x of the K_D of bevacizumab or of ranibizumab, a K_D of 0.7x of the K_D of bevacizumab or of ranibizumab, a K_D of 0.8x of the K_D of bevacizumab or of ranibizumab, a K_D of 0.9x of the K_D of bevacizumab or of ranibizumab, a K_D of 1x of the K_D of bevacizumab or of ranibizumab, a K_D of 1.5x of the K_D of bevacizumab or of ranibizumab, a K_D of 2x of the K_D of bevacizumab or of ranibizumab, a K_D of 4x of the K_D of bevacizumab or of ranibizumab, a K_D of 7.5x of the K_D of bevacizumab or of ranibizumab, a K_D of 10x of the K_D of bevacizumab or of ranibizumab or a K_D ranging between any pair of the foregoing values, *e.g.*, a K_D of 0.01x-2x of the K_D of bevacizumab or of ranibizumab, a K_D of 0.1x-1.5x of the K_D of bevacizumab or of ranibizumab, a K_D of 0.7x-4x of the K_D of bevacizumab or of ranibizumab, a K_D of 0.2x-2x of the K_D of bevacizumab or of ranibizumab or any value or range that can be calculated from the k_{on} and k_{off} rates disclosed herein.

[0121] In some embodiments, an anti-VEGF antibody of the disclosure binds to VEGF and inhibits the binding of VEGF to Flt-1, Flk-1 or both, or neutralize the activity of VEGF at an IC_{50} value ranging from 0.001x to 10x of the IC_{50} of bevacizumab or of ranibizumab, for example an IC_{50} of 0.001x of the IC_{50} of bevacizumab or of ranibizumab, an IC_{50} of 0.005x of the IC_{50} of bevacizumab or of ranibizumab, an IC_{50} of 0.01x of the IC_{50} of bevacizumab or of ranibizumab, an IC_{50} of 0.05x of the IC_{50} of bevacizumab or of ranibizumab, an IC_{50} of 0.075x of the IC_{50} of bevacizumab or of ranibizumab, an IC_{50} of 0.1x of the IC_{50} of bevacizumab or of ranibizumab, an IC_{50} of 0.2x of the IC_{50} of bevacizumab or of ranibizumab, an IC_{50} of 0.3x of the IC_{50} of bevacizumab or of ranibizumab, an IC_{50} of 0.4x of the IC_{50} of bevacizumab or of ranibizumab, an $IC_{50}$of 0.5x of the IC_{50} of bevacizumab or of ranibizumab, an IC_{50} of 0.6x of the IC_{50} of bevacizumab or of ranibizumab, an IC_{50} of 0.7x of the IC_{50} of bevacizumab or of ranibizumab, an IC_{50} of 0.8x of the IC_{50} of bevacizumab or of ranibizumab, an IC_{50} of 0.9x of the IC_{50} of bevacizumab or of ranibizumab, an IC_{50} of 1x of the IC_{50} of bevacizumab or of ranibizumab, an IC_{50} of 1.5x of the IC_{50} of bevacizumab or of ranibizumab, an IC_{50} of 2x of the IC_{50} of bevacizumab or of ranibizumab, an IC_{50} of 4x of the IC_{50} of bevacizumab or of ranibizumab, an IC_{50} of 7.5x of the IC_{50} of bevacizumab or of ranibizumab, an IC_{50} of 10x of the IC_{50} of bevacizumab or of ranibizumab or an IC_{50} ranging between any pair of the foregoing values, *e.g.*, an IC_{50} of 0.01x-2x of the IC_{50} of bevacizumab or of ranibizumab, an IC_{50} of 0.1x-1.5x of the IC_{50} of bevacizumab or of ranibizumab, an IC_{50} of 0.7x-4x of the IC_{50} of bevacizumab or of ranibizumab, an IC_{50} of 0.2x-2x of the IC_{50} of bevacizumab or of ranibizumab. In certain embodiments, a single CDR substitution can result in the foregoing differences in IC_{50} as compared to bevacizumab or ranibizumab, whereas an anti-VEGF antibody of the disclosure can comprise such substitution and up to 16 additional CDR substitutions as compared to bevacizumab or ranibizumab.

6.5 REDUCED IMMUNOGENICITY OF ANTI-VEGF ANTIBODIES

[0122] In certain aspects, the present disclosure provides anti-VEGF antibodies having reduced immunogenicity as compared to bevacizumab or ranibizumab. The present disclosure provides anti-VEGF antibodies having single or multiple amino acid substitutions in their CDRs and/or framework regions as compared to the CDRs of bevacizumab, wherein at least one substitution reduces the immunogenicity of the antibody as compared to bevacizumab or ranibizumab. In certain embodiments, the reduced immunogenicity results from one or more amino acid substitutions that result in eliminating or mitigating one or more T cell epitopes.

[0123] In certain aspects, the anti-VEGF antibodies of the disclosure having reduced immunogenicity have comparable or improved biological activity as compared to bevacizumab or ranibizumab, *e.g.*, affinity towards VEGF or neutralization of VEGF activity. Such properties can be tested, for example, by the methods described in Section 6.3 above.

[0124] In certain embodiments, the immunogenicity of an anti-VEGF antibody of the disclosure is reduced relative to bevacizumab or ranibizumab antibody. Such antibodies generally have variant sequences relative to the heavy and/or light chain variable region in regions corresponding to SEQ ID NO:25, SEQ ID NO:62 and/or SEQ ID NO:74. The antibodies will generally have one, two or three amino acid substitutions in one, two or all three sequences corresponding to SEQ ID NO:25, SEQ ID NO:62, and SEQ ID NO:74, although up to four or five substitutions in one, two or all three regions are contemplated herein.

[0125] As used in the present disclosure, a variant with "reduced immunogenicity" refers to an anti-VEGF antibody with a variant sequence in a region corresponding to SEQ ID NO:25, SEQ ID NO:62, and/or SEQ ID NO:74 that elicits a reduced proliferative response in peripheral blood mononuclear cells as compared to a peptide of SEQ ID NO:25, SEQ ID NO:62, or SEQ ID NO:74, respectively. An exemplary proliferation assay that can be used to evaluate the proliferative response is set forth in Section 7 below. The reduced proliferative response can be reflected in terms of the percentage of responders, the stimulation index, or both.

[0126] In other embodiments, as compared to a peptide having the sequence of SEQ ID NO:25, SEQ ID NO:62, or SEQ ID NO:74, the variant sequence results in at least 25% fewer responders, in at least 30% fewer responders, in at least 35% fewer responders, in at least 40% fewer responders, in at least 45% fewer responders, in at least 50% fewer responders, in at least 60% fewer responders, in at least 65% fewer responders, in at least 70% fewer responders, in at least 75% fewer responders, in at least 80% fewer responders, in at least 85% fewer responders, in at least 90% fewer responders, in at least 95% fewer responders, 100% fewer responders, or a reduction in responders in a range between any of the foregoing values, *e.g.*, 25%-75% fewer responders, 50%-90% fewer responders, 60%-100% fewer responders, 70%-90% fewer responders, or the like.

[0127] In other embodiments, the variant sequence results in a stimulation index that is at least 5% less, at least 10% less, at least 15% less, at least 20% less, at least 25% less, at least 30% less, at least 35% less, or at least 40% less than the stimulation index elicited by a peptide of SEQ ID NO:25, SEQ ID NO:62, or SEQ ID NO:74, respectively, or results in a stimulation index reduced by a range between any of the foregoing values as compared to a peptide of SEQ ID NO:25, SEQ ID NO:62, or SEQ ID NO:74, *e.g.*, 5%-20% less, 10%-30% less, 25%-35% less, 30%-40% less, or the like.

[0128] Exemplary embodiments of candidate anti-VEGF antibodies with reduced immunogenicity as compared to bevacizumab or ranibizumab comprise one or more of the CDR substitutions or combinations of substitutions set forth in Table 6. Optionally, anti-VEGF antibodies with reduced immunogenicity as compared to bevacizumab or ranibizumab comprise one or more additional substitutions, such as the CDR mutations in any of Tables 7-13, singly or in combination.

[0129] Yet further exemplary embodiments of candidate anti-VEGF antibodies with reduced immunogenicity as compared to bevacizumab or ranibizumab comprise one or more of the CDR substitutions or combinations of substitutions set forth in Tables 14–16.

Some preferred embodiments of anti-VEGF antibodies with reduced immunogenicity as compared to bevacizumab or ranibizumab are provided in Table 19.

6.6 ANTIBODY CONJUGATES

[0130] The anti-VEGF antibodies of the disclosure include antibody conjugates that are modified, *e.g.*, by the covalent attachment of any type of molecule to the antibody, such that covalent attachment does not interfere with binding to VEGF.

[0131] In certain aspects, an anti-VEGF antibody of the disclosure can be conjugated to an effector moiety or a label. The term "effector moiety" as used herein includes, for example, antineoplastic agents, drugs, toxins, biologically active proteins, for example enzymes, other antibody or antibody fragments, synthetic or naturally occurring polymers, nucleic acids (*e.g.*, DNA and RNA), radionuclides, particularly radioiodide, radioisotopes, chelated metals, nanoparticles and reporter groups such as fluorescent compounds or compounds which can be detected by NMR or ESR spectroscopy.

[0132] In one example, anti-VEGF antibodies can be conjugated to an effector moiety, such as a cytotoxic agent, a radionuclide or drug moiety to modify a given biological response. The effector moiety can be a protein or polypeptide, such as, for example and without limitation, a toxin (such as abrin, ricin A, *Pseudomonas* exotoxin, or *Diphtheria* toxin), a signaling molecule (such as α-interferon, β-interferon, nerve growth factor, platelet derived growth factor or tissue plasminogen activator), a thrombotic agent or an anti-angiogenic agent (*e.g.*, angiostatin or endostatin) or a biological response modifier such as a cytokine or growth factor (*e.g.*, interleukin-1 (IL-I), interleukin-2 (IL-2), interleukin-6 (IL-6), granulocyte macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), or nerve growth factor (NGF)).

[0133] In another example the effector moieties can be cytotoxins or cytotoxic agents. Examples of cytotoxins and cytotoxic agents include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorabicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof.

[0134] Effector moieties also include, but are not limited to, antimetabolites (*e.g.* methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (*e.g.*, mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C5 and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (*e.g.*, daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (*e.g.*, dactinomycin (formerly actinomycin), bleomycin, mithramycin, anthramycin (AMC), calicheamicins or duocarmycins), and anti-mitotic agents (*e.g.*, vincristine and vinblastine).

[0135] Other effector moieties can include radionuclides such as, but not limited to, ^{111}In and ^{90}Y, Lu^{177}, Bismuth^{213}, Californium^{252}, Iridium^{192} and Tungsten^{188}/Rhenium^{188} and drugs such as, but not limited to, alkylphosphocholines, topoisomerase I inhibitors, taxoids and suramin.

[0136] Techniques for conjugating such effector moieties to antibodies are well known in the art (see, *e.g.*, Hellstrom *et al.,* Controlled Drug Delivery, 2nd Ed., at pp. 623-53 (Robinson *et al.,* eds., 1987)); Thorpe *et al.,* 1982, Immunol. Rev. 62:119-58 and Dubowchik *et al.,* 1999, Pharmacology and Therapeutics 83:67-123).

[0137] In one example, the anti-VEGF antibody or fragment thereof is fused via a covalent bond (*e.g.*, a peptide bond), through the antibody's N-terminus or C-terminus or internally, to an amino acid sequence of another protein (or portion thereof; for example at least a 10, 20 or 50 amino acid portion of the protein). The antibody, or fragment thereof, can linked to the other protein at the N-terminus of the constant domain of the antibody. Recombinant DNA procedures can be used to create such fusions, for example as described in WO 86/01533 and EP0392745. In another example the effector molecule can increase half-life *in vivo*, and/or enhance the delivery of an antibody across an epithelial barrier to the immune system. Examples of suitable effector molecules of this type include polymers, albumin, albumin binding proteins or albumin binding compounds such as those described in WO 2005/117984.

[0138] In certain aspects, an anti-VEGF antibody is conjugated to a small molecule toxin. In certain exemplary embodiments, an anti-VEGF antibody of the disclosure is conjugated to a dolastatin or a dolostatin peptidic analogs or derivatives, *e.g.*, an auristatin

(U.S. Pat. Nos. 5,635,483 and 5,780,588). The dolastatin or auristatin drug moiety may be attached to the antibody through its N (amino) terminus, C (carboxyl) terminus or internally (WO 02/088172). Exemplary auristatin embodiments include the N-terminus linked monomethylauristatin drug moieties DE and DF, as disclosed in U.S. Patent No. 7,498,298, which is hereby incorporated by reference in its entirety (disclosing, *e.g.*, linkers and methods of preparing monomethylvaline compounds such as MMAE and MMAF conjugated to linkers).

[0139] In other exemplary embodiments, small molecule toxins include but are not limited to calicheamicin, maytansine (U.S. Pat. No. 5,208,020), trichothene, and CC1065. In one embodiment of the disclosure, the antibody is conjugated to one or more maytansine molecules (*e.g.*, about 1 to about 10 maytansine molecules per antibody molecule). Maytansine may, for example, be converted to May-SS-Me which may be reduced to May-SH3 and reacted with an antibody (Chari *et al.*, 1992, Cancer Research 52: 127-131) to generate a maytansinoid-antibody or maytansinoid-Fc fusion conjugate. Structural analogues of calicheamicin that can also be used include but are not limited to γ_1^1, γ_3^1, N-acetyl- γ_1^1, PSAG, and θ_1^1, (Hinman *et al.*, 1993, Cancer Research 53:3336-3342; Lode *et al.*, 1998, Cancer Research 58:2925-2928; U.S. Patent No. 5,714,586; U.S. Patent No. 5,712,374; U.S. Patent No. 5,264,586; U.S. Patent No. 5,773,001).

[0140] Antibodies of the disclosure can also be conjugated to liposomes for targeted delivery (See, *e.g.*, Park *et al.*, 1997, Adv. Pharmacol. 40:399–435; Marty & Schwendener, 2004, Methods in Molecular Medicine 109:389-401).

[0141] In one example antibodies of the present disclosure can be attached to poly(ethyleneglycol) (PEG) moieties. In one particular example the antibody is an antibody fragment and the PEG moieties can be attached through any available amino acid side-chain or terminal amino acid functional group located in the antibody fragment, for example any free amino, imino, thiol, hydroxyl or carboxyl group. Such amino acids can occur naturally in the antibody fragment or can be engineered into the fragment using recombinant DNA methods. See for example U.S. Patent No. 5,219,996. Multiple sites can be used to attach two or more PEG molecules. PEG moieties can be covalently linked through a thiol group of at least one cysteine residue located in the antibody fragment. Where a thiol group is used as the point of attachment, appropriately activated effector moieties, for example thiol selective derivatives such as maleimides and cysteine derivatives, can be used.

[0142] In a specific example, an anti-VEGF antibody conjugate is a modified Fab' fragment which is PEGylated, *i.e.*, has PEG (poly(ethyleneglycol)) covalently attached thereto, *e.g.*, according to the method disclosed in EP0948544. See also Poly(ethyleneglycol) Chemistry, Biotechnical and Biomedical Applications, (J. Milton Harris (ed.), Plenum Press, New York, 1992); Poly(ethyleneglycol) Chemistry and Biological Applications, (J. Milton Harris and S. Zalipsky, eds., American Chemical Society, Washington D.C., 1997); and Bioconjugation Protein Coupling Techniques for the Biomedical Sciences, (M. Aslam and A. Dent, eds., Grove Publishers, New York, 1998); and Chapman, 2002, Advanced Drug Delivery Reviews 54:531-545. PEG can be attached to a cysteine in the hinge region. In one example, a PEG-modified Fab' fragment has a maleimide group covalently linked to a single thiol group in a modified hinge region. A lysine residue can be covalently linked to the maleimide group and to each of the amine groups on the lysine residue can be attached a methoxypoly(ethyleneglycol) polymer having a molecular weight of approximately 20,000 Da. The total molecular weight of the PEG attached to the Fab' fragment can therefore be approximately 40,000 Da.

[0143] The word “label” when used herein refers to a detectable compound or composition which can be conjugated directly or indirectly to an anti-VEGF antibody of the disclosure. The label can itself be detectable (*e.g.*, radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, can catalyze chemical alteration of a substrate compound or composition which is detectable. Useful fluorescent moieties include, but are not limited to, fluorescein, fluorescein isothiocyanate, rhodamine, 5-dimethylamine-1-napthalenesulfonyl chloride, phycoerythrin and the like. Useful enzymatic labels include, but are not limited to, alkaline phosphatase, horseradish peroxidase, glucose oxidase and the like.

[0144] Additional anti-VEGF antibody conjugates that are useful for, *inter alia*, diagnostic purposes, are described in Section 6.7 below.

6.7 DIAGNOSTIC USES OF ANTI-VEGF ANTIBODIES

[0145] The anti-VEGF antibodies of the disclosure, including those antibodies that have been modified, *e.g.*, by biotinylation, horseradish peroxidase, or any other detectable moiety (including those described in Section 6.6), can be advantageously used for diagnostic purposes.

[0146] In particular, the anti-VEGF antibodies can be used, for example, but not limited to, to purify or detect VEGF, including both *in vitro* and *in vivo* diagnostic methods. For example, the antibodies have use in immunoassays for qualitatively and quantitatively measuring levels of VEGF in biological samples. *See*, *e.g.*, Harlow *et al.,* Antibodies: A Laboratory Manual, Second Edition (Cold Spring Harbor Laboratory Press, 1988), which is incorporated by reference herein in its entirety.

[0147] The present disclosure further encompasses antibodies or fragments thereof conjugated to a diagnostic agent. The antibodies can be used diagnostically, for example, to detect expression of a target of interest in specific cells, tissues, or serum; or to monitor the development or progression of an immunologic response as part of a clinical testing procedure to, *e.g.,* determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive materials, positron emitting metals using various positron emission tomographies, and nonradioactive paramagnetic metal ions. The detectable substance can be coupled or conjugated either directly to the antibody (or fragment thereof) or indirectly, through an intermediate (such as, for example, a linker known in the art) using techniques known in the art. Examples of enzymatic labels include luciferases (*e.g.*, firefly luciferase and bacterial luciferase; U.S. Patent No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, malate dehydrogenase, urease, peroxidase such as horseradish peroxidase (HRPO), alkaline phosphatase, β-galactosidase, acetylcholinesterase, glucoamylase, lysozyme, saccharide oxidases (*e.g.*, glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase), heterocyclic oxidases (such as uricase and xanthine oxidase), lactoperoxidase, microperoxidase, and the like. Examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include ^{125}I, ^{131}I, ^{111}In or ^{99}Tc.

[0148] The disclosure provides for the detection of expression of VEGF, comprising contacting a biological sample (cells, tissue, or body fluid of an individual) using one or more anti-VEGF antibodies of the disclosure (optionally conjugated to detectable moiety), and detecting whether or not the sample is positive for VEGF expression, or whether the sample has altered (*e.g.*, reduced or increased) expression as compared to a control sample.

[0149] Diseases that can be diagnosed using the present methods include, but are not limited to, the diseases described herein. In certain embodiments, the tissue or body fluid is peripheral blood, peripheral blood leukocytes, biopsy tissues such as lung or skin biopsies, and tissue.

6.8 THERAPEUTIC METHODS USING ANTI-VEGF ANTIBODIES

6.8.1 Clinical Benefits

[0150] The anti-VEGF antibodies of the disclosure can be used to treat various neoplasms or non-neoplastic conditions characterized by pathological angiogenesis.

[0151] The antibodies of the disclosure are useful in the treatment of tumors in which angiogenesis plays an important role in tumor growth, including cancers and benign tumors. Examples of cancer to be treated herein include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia. More particular examples of such cancers include squamous cell cancer, lung cancer (including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and squamous carcinoma of the lung), cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer (including gastrointestinal cancer), pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma and various types of head and neck cancer, as well as B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL)

NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; and Waldenstrom's Macroglobulinemia); chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); Hairy cell leukemia; chronic myeloblastic leukemia; and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phakomatoses, edema (such as that associated with brain tumors), and Meigs' syndrome. More particularly, cancers that are amenable to treatment by the antibodies of the disclosure include breast cancer, colorectal cancer, rectal cancer, non-small cell lung cancer, non-Hodgkins lymphoma (NHL), renal cell cancer, prostate cancer, liver cancer, pancreatic cancer, soft-tissue sarcoma, kaposi's sarcoma, carcinoid carcinoma, head and neck cancer, melanoma, ovarian cancer, mesothelioma, and multiple myeloma. In some embodiments, the anti-VEGF antibodies of the disclosure are used to treat colorectal cancer in a human patient.

[0152] The present disclosure encompasses anti-angiogenic therapy, a cancer treatment strategy aimed at inhibiting the development of tumor blood vessels required for providing nutrients to support tumor growth. Because angiogenesis is involved in both primary tumor growth and metastasis, the antiangiogenic treatment provided by the disclosure is capable of inhibiting the neoplastic growth of tumor at the primary site as well as preventing metastasis of tumors at the secondary sites.

[0153] Non-neoplastic conditions that are amenable to treatment with the antibodies of the disclosure include retinal diseases (*e.g.*, age-related macular degeneration) and immune and inflammatory diseases (*e.g.*, rheumatoid arthritis, psoriasis, atherosclerosis, diabetic and other proliferative retinopathies including retinopathy of prematurity, retrolental fibroplasia, neovascular glaucoma, age-related macular degeneration, thyroid hyperplasias (including Grave's disease), corneal and other tissue transplantation, chronic inflammation, lung inflammation, nephrotic syndrome, preeclampsia, ascites, pericardial effusion (such as that associated with pericarditis), and pleural effusion).

[0154] Accordingly, the present disclosure provides methods of treating any of the foregoing diseases in a patient in need thereof, comprising: administering to the patient an anti-VEGF antibody of the disclosure. Optionally, said administration is repeated, *e.g.*, after one day, two days, three days, five days, one week, two weeks, three weeks, one month, five weeks, six weeks, seven weeks, eight weeks, two months or three months. The repeated administration can be at the same dose or at a different dose. The administration can be repeated once, twice, three times, four times, five times, six times, seven times, eight times, nine times, ten times, or more. For example, according to certain dosage regimens a patient receives anti-VEGF therapy for a prolonged period of time, *e.g.*, 6 months, 1 year or more. The amount of anti-VEGF antibody administered to the patient is in certain embodiments a therapeutically effective amount. As used herein, a “therapeutically effective” amount of VEGF antibody can be administered as a single dose or over the course of a therapeutic regimen, *e.g.*, over the course of a week, two weeks, three weeks, one month, three months, six months, one year, or longer. Exemplary therapeutic regimens are described in Section 6.11 below.

[0155] According to the present disclosure, treatment of a disease encompasses the treatment of patients already diagnosed as having any form of the disease at any clinical stage or manifestation; the delay of the onset or evolution or aggravation or deterioration of the symptoms or signs of the disease; and/or preventing and/or reducing the severity of the disease.

[0156] A “subject” or “patient” to whom the anti-VEGF antibody of the disclosure is administered is preferably a mammal such as a non-primate (*e.g.*, cow, pig, horse, cat, dog, rat, *etc.*) or a primate (*e.g.*, monkey or human). In certain embodiments, the subject or patient is a human. In certain aspects, the human is a pediatric patient. In other aspects, the human is an adult patient.

6.9 PHARMACEUTICAL COMPOSITIONS AND ROUTES OF ADMINISTRATION

[0157] Compositions comprising an anti-VEGF antibody of the disclosure and, optionally one or more additional therapeutic agents, such as the combination therapeutic agents described in Section 6.10 below, are provided herein. The compositions will usually be supplied as part of a sterile, pharmaceutical composition that will normally include a pharmaceutically acceptable carrier. This composition can be in any suitable form (depending upon the desired method of administering it to a patient).

[0158] The anti-VEGF antibodies of the disclosure can be administered to a patient by a variety of routes such as orally, transdermally, subcutaneously, intranasally, intravenously, intramuscularly, intraocularly, topically, intrathecally and intracerebroventricularly. The most suitable route for administration in any given case will depend on the particular antibody, the subject, and the nature and severity of the disease and the physical condition of the subject.

[0159] For treatment of indications other than retinal diseases, the effective dose of an anti-VEGF antibody of the disclosure can range from about 0.1 to about 75 mg/kg per single (*e.g.*, bolus) administration, multiple administrations or continuous administration, or to achieve a serum concentration of 0.01-5000 μg/mL serum concentration per single (*e.g.*, bolus) administration, multiple administrations or continuous administration, or any effective range or value therein depending on the condition being treated, the route of administration and the age, weight and condition of the subject. In certain embodiments, *e.g.* for the treatment of cancer, each dose can range from about 0.1 mg to about 50 mg per kilogram of body weight, for example from about 3 mg to about 25 mg per kilogram body weight. The antibody can be formulated as an aqueous solution and administered by subcutaneous injection.

[0160] For treatment of retinal diseases (*e.g.*, age-related macular degeneration (AMD), the dosage suitably results in aqueous humor concentration of the anti-VEGF antibody the injected eye of 1-50 μg/mL. For treatment of AMD, each dose can be from 0.1 mg to about 1 mg, for example from about 0.25 to about 0.5 mg. The antibody can be formulated as an aqueous solution and administered by intravitreal injection.

[0161] Pharmaceutical compositions can be conveniently presented in unit dose forms containing a predetermined amount of an anti-VEGF antibody of the disclosure per dose. Such a unit can contain for example but without limitation 0.1 mg to 5 g, for example 1 mg to 1 g, or 10 to 50 mg. Pharmaceutically acceptable carriers for use in the disclosure can take a wide variety of forms depending, *e.g.*, on the condition to be treated or route of administration.

[0162] Therapeutic formulations of the anti-VEGF antibodies of the disclosure can be prepared for storage as lyophilized formulations or aqueous solutions by mixing the antibody having the desired degree of purity with optional pharmaceutically-acceptable carriers, excipients or stabilizers typically employed in the art (all of which are referred to herein as “carriers”), *i.e.*, buffering agents, stabilizing agents, preservatives, isotonifiers, non-ionic detergents, antioxidants, and other miscellaneous additives. See, Remington’s Pharmaceutical Sciences, 16th edition (Osol, ed. 1980). Such additives must be nontoxic to the recipients at the dosages and concentrations employed.

[0163] Buffering agents help to maintain the pH in the range which approximates physiological conditions. They can be present at concentration ranging from about 2 mM to about 50 mM. Suitable buffering agents for use with the present disclosure include both organic and inorganic acids and salts thereof such as citrate buffers (*e.g.*, monosodium citrate-disodium citrate mixture, citric acid-trisodium citrate mixture, citric acid-monosodium citrate mixture, *etc.*), succinate buffers (*e.g.*, succinic acid-monosodium succinate mixture, succinic acid-sodium hydroxide mixture, succinic acid-disodium succinate mixture, *etc.*), tartrate buffers (*e.g.*, tartaric acid-sodium tartrate mixture, tartaric acid-potassium tartrate mixture, tartaric acid-sodium hydroxide mixture, *etc.*), fumarate buffers (*e.g.*, fumaric acid-monosodium fumarate mixture, fumaric acid-disodium fumarate mixture, monosodium fumarate-disodium fumarate mixture, *etc.*), gluconate buffers (*e.g.*, gluconic acid-sodium glyconate mixture, gluconic acid-sodium hydroxide mixture, gluconic acid-potassium glyuconate mixture, *etc.*), oxalate buffer (*e.g.*, oxalic acid-sodium oxalate mixture, oxalic acid-sodium hydroxide mixture, oxalic acid-potassium oxalate mixture, *etc.*), lactate buffers (*e.g.*, lactic acid-sodium lactate mixture, lactic acid-sodium hydroxide mixture, lactic acid-potassium lactate mixture, *etc.*) and acetate buffers (*e.g.*, acetic acid-sodium acetate mixture, acetic acid-sodium hydroxide mixture, *etc.*). Additionally, phosphate buffers, histidine buffers and trimethylamine salts such as Tris can be used.

[0164] Preservatives can be added to retard microbial growth, and can be added in amounts ranging from 0.2%-1% (w/v). Suitable preservatives for use with the present disclosure include phenol, benzyl alcohol, meta-cresol, methyl paraben, propyl paraben, octadecyldimethylbenzyl ammonium chloride, benzalconium halides (*e.g.*, chloride, bromide, and iodide), hexamethonium chloride, and alkyl parabens such as methyl or propyl paraben, catechol, resorcinol, cyclohexanol, and 3-pentanol. Isotonicifiers sometimes known as “stabilizers” can be added to ensure isotonicity of liquid compositions of the present disclosure and include polhydric sugar alcohols, for example trihydric or higher sugar alcohols, such as glycerin, erythritol, arabitol, xylitol, sorbitol and mannitol. Stabilizers refer to a broad category of excipients which can range in function from a bulking agent to an additive which solubilizes the therapeutic agent or helps to prevent denaturation or adherence to the container wall. Typical stabilizers can be polyhydric sugar alcohols (enumerated above); amino acids such as arginine, lysine, glycine, glutamine, asparagine, histidine, alanine, ornithine, L-leucine, 2-phenylalanine, glutamic acid, threonine, *etc.*, organic sugars or sugar alcohols, such as lactose, trehalose, stachyose, mannitol, sorbitol, xylitol, ribitol, myoinisitol, galactitol, glycerol and the like, including cyclitols such as inositol; polyethylene glycol; amino acid polymers; sulfur containing reducing agents, such as urea, glutathione, thioctic acid, sodium thioglycolate, thioglycerol, α-monothioglycerol and sodium thio sulfate; low molecular weight polypeptides (*e.g.*, peptides of 10 residues or fewer); proteins such as human serum albumin, bovine serum albumin, gelatin or immunoglobulins; hydrophylic polymers, such as polyvinylpyrrolidone monosaccharides, such as xylose, mannose, fructose, glucose; disaccharides such as lactose, maltose, sucrose and trisaccacharides such as raffinose; and polysaccharides such as dextran. Stabilizers can be present in the range from 0.1 to 10,000 weights per part of weight active protein.

[0165] Non-ionic surfactants or detergents (also known as "wetting agents") can be added to help solubilize the therapeutic agent as well as to protect the therapeutic protein against agitation-induced aggregation, which also permits the formulation to be exposed to shear surface stressed without causing denaturation of the protein. Suitable non-ionic surfactants include polysorbates (20, 80, *etc.*), polyoxamers (184, 188 *etc.*), Pluronic polyols, polyoxyethylene sorbitan monoethers (TWEEN®-20, TWEEN®-80, *etc.*). Non-ionic surfactants can be present in a range of about 0.05 mg/mL to about 1.0 mg/mL, for example about 0.07 mg/mL to about 0.2 mg/mL.

[0166] Additional miscellaneous excipients include bulking agents (*e.g.*, starch), chelating agents (*e.g.*, EDTA), antioxidants (*e.g.*, ascorbic acid, methionine, vitamin E), and cosolvents.

[0167] The formulation herein can also contain a combination therapeutic agent in addition to the anti-VEGF antibody of the disclosure. Examples of suitable combination therapeutic agents are provided in Section 6.10 below.

[0168] The dosing schedule for subcutaneous administration can vary from once everyt six months to daily depending on a number of clinical factors, including the type of disease, severity of disease, and the patient's sensitivity to the anti-VEGF antibody.

[0169] The dosage of an anti-VEGF antibody of the disclosure to be administered of will vary according to the particular antibody, the type of disease (*e.g.*, cancer, inflammatory, etc.), the subject, and the severity of the disease, the physical condition of the subject, the therapeutic regimen (*e.g.*, whether a combination therapeutic agent is used), and the selected route of administration; the appropriate dosage can be readily determined by a person skilled in the art.

[0170] It will be recognized by one of skill in the art that the optimal quantity and spacing of individual dosages of an anti-VEGF antibody of the disclosure will be determined by the nature and extent of the condition being treated, the form, route and site of administration, and the age and condition of the particular subject being treated, and that a physician will ultimately determine appropriate dosages to be used. This dosage can be repeated as often as appropriate. If side effects develop the amount and/or frequency of the dosage can be altered or reduced, in accordance with normal clinical practice.

6.10 COMBINATION THERAPY

[0171] Described below are combinatorial methods in which the anti-VEGF antibodies of the disclosure can be utilized. The combinatorial methods of the disclosure involve the administration of at least two agents to a patient, the first of which is an anti-VEGF antibody of the disclosure, and the second of which is a combination therapeutic agent. The anti-VEGF antibody and the combination therapeutic agent can be administered simultaneously, sequentially or separately.

[0172] The combinatorial therapy methods of the present disclosure can result in a greater than additive effect, providing therapeutic benefits where neither the anti-VEGF antibody or combination therapeutic agent administered in an amount that is alone therapeutically effective.

[0173] In the present methods, the anti-VEGF antibody of the disclosure and the combination therapeutic agent can be administered concurrently, either simultaneously or successively. As used herein, the anti-VEGF antibody of the disclosure and the combination therapeutic agent are said to be administered successively if they are administered to the patient on the same day, for example during the same patient visit. Successive administration can occur 1, 2, 3, 4, 5, 6, 7 or 8 hours apart. In contrast, the anti-VEGF antibody of the disclosure and the combination therapeutic agent are said to be administered separately if they are administered to the patient on the different days, for example, the anti-VEGF antibody of the disclosure and the combination therapeutic agent can be administered at a 1-day, 2-day or 3-day, one-week, 2-week or monthly intervals. In the methods of the present disclosure, administration of the anti-VEGF antibody of the disclosure can precede or follow administration of the combination therapeutic agent.

[0174] As a non-limiting example, the anti-VEGF antibody of the disclosure and combination therapeutic agent can be administered concurrently for a period of time, followed by a second period of time in which the administration of the anti-VEGF antibody of the disclosure and the combination therapeutic agent is alternated.

[0175] Because of the potentially synergistic effects of administering an anti-VEGF antibody of the disclosure and a combination therapeutic agent, such agents can be administered in amounts that, if one or both of the agents is administered alone, is/are not therapeutically effective.

[0176] In certain aspects, the combination therapeutic agent is an anti-angiogenic agent, an anti-rheumatic drug, an anti-inflammatory agent, a chemotherapeutic agent, a radiotherapeutic, an immunosuppressive agent, or a cytotoxic drug.

[0177] It is contemplated that when used to treat various diseases, the anti-VEGF antibodies of the disclosure can be combined with other therapeutic agents suitable for the same or similar diseases. When used for treating cancer, antibodies of the present disclosure may be used in combination with conventional cancer therapies, such as surgery, radiotherapy, chemotherapy or combinations thereof.

[0178] In some other aspects, other therapeutic agents useful for combination tumor therapy with the antibody of the disclosure include antagonists, *e.g.*, antibodies, of other factors that are involved in tumor growth, such as EGFR, ErbB2 (also known as Her2), ErbB3, ErbB4, or TNF-α.

[0179] Sometimes, for treatment of cancers and immune diseases, it may be beneficial to also administer one or more cytokines to the patient. In a preferred embodiment, the VEGF antibody is co-administered with a growth inhibitory agent.

[0180] Suitable dosages for the growth inhibitory agent are those presently used and may be lowered due to the combined action (synergy) of the growth inhibitory agent and anti-VEGF antibody.

[0181] For treatment of cancers, immune diseases and retinal diseases, anti-inflammatory agents can suitably be used in combination with the anti-VEGF antibodies of the disclosure. Anti-inflammatory agents include, but are not limited to, acetaminophen, diphenhydramine, meperidine, dexamethasone, pentasa, mesalazine, asacol, codeine phosphate, benorylate, fenbufen, naprosyn, diclofenac, etodolac and indomethacin, aspirin and ibuprofen.

[0182] For treatment of cancers, chemotherapeutic agents can suitably be used in combination with the anti-VEGF antibodies of the disclosure. Chemotherapeutic agents include, but are not limited to, radioactive molecules, toxins, also referred to as cytotoxins or cytotoxic agents, which includes any agent that is detrimental to the viability of cells, agents, and liposomes or other vesicles containing chemotherapeutic compounds. Examples of suitable chemotherapeutic agents include but are not limited to 1-dehydrotestosterone, 5-fluorouracil decarbazine, 6-mercaptopurine, 6-thioguanine, actinomycin D, adriamycin, aldesleukin, an anti-α5β1 integrin antibody, alkylating agents, allopurinol sodium, altretamine, amifostine, anastrozole, anthramycin (AMC)), anti-mitotic agents, cis-dichlorodiamine platinum (II) (DDP) cisplatin, diamino dichloro platinum, anthracyclines, antibiotics, antimetabolites, asparaginase, BCG live (intravesical), betamethasone sodium phosphate and betamethasone acetate, bicalutamide, bleomycin sulfate, busulfan, calcium leucouorin, calicheamicin, capecitabine, carboplatin, lomustine (CCNU), carmustine (BSNU), Chlorambucil, Cisplatin, Cladribine, Colchicin, conjugated estrogens, Cyclophosphamide, Cyclothosphamide, Cytarabine, Cytarabine, cytochalasin B, Cytoxan, Dacarbazine, Dactinomycin, dactinomycin (formerly actinomycin), daunirubicin HCL, daunorucbicin citrate, denileukin diftitox, Dexrazoxane, Dibromomannitol, dihydroxy anthracin dione, Docetaxel, dolasetron mesylate, doxorubicin HCL, dronabinol, *E. coli* L-asparaginase, eolociximab, emetine, epoetin-α, Erwinia L-asparaginase, esterified estrogens, estradiol, estramustine phosphate sodium, ethidium bromide, ethinyl estradiol, etidronate, etoposide citrororum factor, etoposide phosphate, filgrastim, floxuridine, fluconazole, fludarabine phosphate, fluorouracil, flutamide, folinic acid, gemcitabine HCL, glucocorticoids, goserelin acetate, gramicidin D, granisetron HCL, hydroxyurea, idarubicin HCL, ifosfamide, interferon α-2b, irinotecan HCL, letrozole, leucovorin calcium, leuprolide acetate, levamisole HCL, lidocaine, lomustine, maytansinoid, mechlorethamine HCL, medroxyprogesterone acetate, megestrol acetate, melphalan HCL, mercaptipurine, mesna, methotrexate, methyltestosterone, mithramycin, mitomycin C, mitotane, mitoxantrone, nilutamide, octreotide acetate, ondansetron HCL, paclitaxel, pamidronate disodium, pentostatin, pilocarpine HCL, plimycin, polifeprosan 20 with carmustine implant, porfimer sodium, procaine, procarbazine HCL, propranolol, rituximab, sargramostim, streptozotocin, tamoxifen, taxol, teniposide, tenoposide, testolactone, tetracaine, thioepa chlorambucil, thioguanine, thiotepa, topotecan HCL, toremifene citrate, trastuzumab, tretinoin, valrubicin, vinblastine sulfate, vincristine sulfate, and vinorelbine tartrate.

[0183] Any anti-angiogenic agent can be used in conjunction with the anti-VEGF antibodies of the disclosure, including those listed by Carmeliet and Jain, 2000, Nature 407:249-257. In certain embodiments, the anti-angiogenic agent is another VEGF antagonist or a VEGF receptor antagonist such as VEGF variants, soluble VEGF receptor fragments, aptamers capable of blocking VEGF or VEGFR, neutralizing anti-VEGFR antibodies, low molecule weight inhibitors of VEGFR tyrosine kinases and any combinations thereof. Alternatively, or in addition, two or more anti-VEGF antibodies may be co-administered to the patient.

[0184] In certain embodiments, hormone therapy can be used in conjunction with anti-VEGF antibodies of the disclosure. In some embodiments, the hormone therapy includes one or more agents that inhibit estrogen and/or progesterone from promoting cancer cell growth, *e.g.,* a selective estrogen-receptor modulator such as tamoxifen, an aromatase inhibitor such as anastrozole (Arimidex®) or letrozole (Femara), an aromatase inactivator such as exemestane (Aromasin®), or an agent that inhibits estrogen production such as goserelin (Zoladex). In other embodiments, the hormone therapy is one or more agents that inhibit production of hormones from the ovaries.

[0185] In some aspects, an anti-VEGF antibody can be used in conjunction with a small molecule protein tyrosine kinase (PTK) inhibitor. In some embodiments, the PTK inhibitor is specific for a VEGF receptor tyrosine kinase. In other embodiments, the PTK inhibitor binds to more than one of the VEGF receptor family of tyrosine kinases (*e.g.,* VEGFR-1, VEGFR-2). In other embodiments, protein tyrosine kinase inhibitors useful in the compositions and methods of the invention include PTK inhibitors that do not bind selectively to the VEGF family of receptor tyrosine kinases, but also bind to the tyrosine kinase domains of other families of proteins such as HER2, HER3, HER4, PDGFR, and/or Raf.

[0186] In some embodiments, the tyrosine kinase is a receptor tyrosine kinase, *i.e.,* is an intra-cellular domain of a larger protein that has an extra-cellular ligand binding domain and is activated by the binding of one or more ligands. In certain embodiments, the protein tyrosine kinase is a non-receptor tyrosine kinase. PTK inhibitors for use in the methods of the present disclosure include, but are not limited to, gefitinib (ZD-1839, Iressa®), erlotinib (OSI-1774, Tarceva™), canertinib (CI-1033), vandetanib (ZD6474, Zactima®), tyrphostin AG-825 (CAS 149092-50-2), lapatinib (GW-572016), sorafenib (BAY43-9006), AG-494 (CAS 133550-35-3), RG-13022 (CAS 149286-90-8), RG-14620 (CAS 136831-49-7), BIBW 2992 (Tovok), tyrphostin 9 (CAS 136831-49-7), tyrphostin 23 (CAS 118409-57-7), tyrphostin 25 (CAS 118409-58-8), tyrphostin 46 (CAS 122520-85-8), tyrphostin 47 (CAS 122520-86-9), tyrphostin 53 (CAS 122520-90-5), butein (1-(2,4-dihydroxyphenyl)-3-(3,4-dihydroxyphenyl)-2-propen-1-one 2′,3,4,4′-Tetrahydroxychalcone; CAS 487-52-5), curcumin ((E,E)-1,7-bis(4-Hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione; CAS 458-37-7), N4-(1-Benzyl-1H-indazol-5-yl)-N6,N6-dimethyl-pyrido-[3,4-d]-pyrimidine-4,6-diamine (202272-68-2), AG-1478, AG-879, Cyclopropanecarboxylic acid-(3-(6-(3-trifluoromethyl-phenylamino)-pyrimidin-4-ylamino)-phenyl)-amide (CAS 879127-07-8), N8-(3-Chloro-4-fluorophenyl)-N2-(1-methylpiperidin-4-yl)-pyrimido[5,4-d]pyrimidine-2,8-diamine, 2HCl (CAS 196612-93-8), 4-(4-Benzyloxyanilino)-6,7-dimethoxyquinazoline (CAS 179248-61-4), N-(4-((3-

Chloro-4-fluorophenyl)amino)pyrido[3,4-d]pyrimidin-6-yl)2-butynamide (CAS 881001-19-0), EKB-569, HKI-272, and HKI-357.

[0187] In a specific embodiment, an anti-VEGF antibody of the disclosure is used in combination with intravenous 5-fluorouracil–based chemotherapy. This combination is suitable for, *inter alia*, first- or second-line treatment of patients with metastatic carcinoma of the colon or rectum.

[0188] In another specific embodiment, an anti-VEGF antibody of the disclosure is used in combination with carboplatin and paclitaxel. This combination is suitable for, *inter alia*, first-line treatment of patients with unresectable, locally advanced, recurrent or metastatic non-squamous, non-small cell lung cancer.

[0189] In yet another specific embodiment, an anti-VEGF antibody of the disclosure is used in combination with paclitaxel. This combination is suitable for, *inter alia*, treatment of patients who have not received chemotherapy for metastatic HER2-negative breast cancer.

[0190] For treatment of retinal diseases, the anti-VEGF antibodies of the disclosure can be used in combination with E10030, an anti-platelet-derived growth factor (PDGF) pegylated aptamer; with ARC1905, a pegylated aptamer targeting the C5 component of the complement cascade; and volociximab, a monoclonal antibody targeting the $\alpha5\beta1$ integrin transmembrane receptor; photodynamic therapy with Visudyne® (PDT); or Macugen®, an aptamer (pegaptanib sodium).

6.11 THERAPEUTIC REGIMENS

[0191] The present disclosure provides therapeutic regimens involving the administration of the anti-VEGF antibodies of the disclosure. The therapeutic regimen will vary depending on the patient's age, weight, and disease condition. The therapeutic regimen can continue for 2 weeks to indefinitely. In specific embodiments, the therapeutic regimen is continued for 2 weeks to 6 months, from 3 months to 5 years, from 6 months to 1 or 2 years, from 8 months to 18 months, or the like. The therapeutic regimen can be a non-variable dose regimen or a multiple-variable dose regimen.

[0192] For the dosage exemplary regimens described below, the anti-VEGF antibody can be administered as a sterile, preservative-free solution for subcutaneous administration.

[0193] For treatment of metastatic colorectal cancer, an anti-VEGF antibody of the disclosure is administered intravenously at a dose of 0.5-15 mg/kg every 2 weeks with bolus-IFL (irinotecan, 5-fluorouracil and leucovorin regimen). In specific embodiments, the dose is 1-4 mg/kg, 2-6 mg/kg, 0.5-3 mg/kg, 1-10 mg/kg, 3-4.8 mg/kg or 1-4.5 mg/kg every two weeks with bolus-IFL.

[0194] In another embodiment for treatment of metastatic colorectal cancer, an anti-VEGF antibody of the disclosure is administered intravenously at a dose of 1-30 mg/kg every 2 weeks with FOLFOX4 (oxaliplatin, leucovorin, and fluorouracil regimen). In specific embodiments, the dose is 2-9 mg/kg, 3-12 mg/kg, 1-7.5 mg/kg, 2-20 mg/kg, 6-9.75 mg/kg or 4-9.5 mg/kg every two weeks with FOLFOX4.

[0195] For treatment of non-squamous non-small cell lung cancer, an anti-VEGF antibody of the disclosure is administered intravenously at a dose of 2-40 mg/kg every three weeks with carboplatin/paclitaxel. In specific embodiments, the dose is 5-14 mg/kg, 4-20 mg/kg, 10-17.5 mg/kg, 7-14 mg/kg, 10-30 mg/kg or 3-30 mg/kg every three weeks with carboplatin/paclitaxel.

[0196] For treatment of metastatic breast cancer, an anti-VEGF antibody of the disclosure is administered intravenously at a dose of 0.5-20 mg/kg every two weeks with paclitaxel. In specific embodiments, the dose is 1-4 mg/kg, 2-6 mg/kg, 0.5-3 mg/kg, 1-10 mg/kg, 3-4.8 mg/kg or 1-4.5 mg/kg every two weeks with paclitaxel.

[0197] For treatment of metastatic breast cancer, an anti-VEGF antibody of the disclosure is administered intravenously at a dose of 0.5-20 mg/kg every two weeks as monotherapy. In specific embodiments, the dose is 1-4 mg/kg, 2-6 mg/kg, 0.5-3 mg/kg, 1-10 mg/kg, 3-4.8 mg/kg or 1-4.5 mg/kg every two weeks as monotherapy.

[0198] For treatment of age-related macular degeneration (AMD), an anti-VEGF antibody of the disclosure is administered at a dose of 0.1-1 mg by intravitreal injection once a month (approximately 28 days). In specific embodiments, the dose is 0.1-0.4 mg, 0.2-0.6 mg, 0.1-0.25 mg, 0.25-0.5 mg, 0.25-0.75 mg, or 0.3-0.45 mg by intravitreal injection once a month (approximately 28 days). In a specific embodiment, a patient treated with an anti-VEGF antibody of the disclosure has wet AMD. In another specific embodiment, a patient has dry AMD.

6.12 DIAGNOSTIC AND PHARMACEUTICAL KITS

[0199] Encompassed by the present disclosure are pharmaceutical kits containing the anti-VEGF antibodies (including antibody conjugates) of the disclosure. The pharmaceutical kit is a package comprising the anti-VEGF antibody of the disclosure (*e.g.*, either in lyophilized form or as an aqueous solution) and one or more of the following:

- A combination therapeutic agent, for example as described in Section 6.10 above;
- A device for administering the anti-VEGF antibody, for example a pen, needle and/or syringe; and
- Pharmaceutical grade water or buffer to resuspend the antibody if the antibody is in lyophilized form.

[0200] In certain aspects, each unit dose of the anti-VEGF antibody is packaged separately, and a kit can contain one or more unit doses (*e.g.*, two unit doses, three unit doses, four unit doses, five unit doses, eight unit doses, ten unit doses, or more). In a specific embodiment, the one or more unit doses are each housed in a syringe or pen.

[0201] Diagnostic kits containing the anti-VEGF antibodies (including antibody conjugates) of the disclosure are also encompassed herein. The diagnostic kit is a package comprising the anti-VEGF antibody of the disclosure (*e.g.*, either in lyophilized form or as an aqueous solution) and one or more reagents useful for performing a diagnostic assay. Where the anti-VEGF antibody is labeled with an enzyme, the kit can include substrates and cofactors required by the enzyme (*e.g.*, a substrate precursor which provides the detectable chromophore or fluorophore). In addition, other additives can be included, such as stabilizers, buffers (*e.g.*, a block buffer or lysis buffer), and the like. In certain embodiments, the anti-VEGF antibody included in a diagnostic kit is immobilized on a solid surface, or a solid surface (*e.g.*, a slide) on which the antibody can be immobilized is included in the kit. The relative amounts of the various reagents can be varied widely to provide for concentrations in solution of the reagents which substantially optimize the sensitivity of the assay. In a specific embodiment, the antibody and one or more reagents can be provided (individually or combined) as dry powders, usually lyophilized, including excipients which on dissolution will provide a reagent solution having the appropriate concentration.

7. EXAMPLE 1: IDENTIFICATION OF T-CELL EPITOPES OF BEVACIZUMAB

7.1 MATERIALS & METHODS

7.1.1 Peptides

[0202] Peptides were synthesized using a multi-pin format by Mimotopes (Adelaide, Australia). The sequences of the bevacizumab light and heavy chain V regions were synthesized as 15-mer peptides overlapping by 12 amino acids (Tables 3 and 4) for a total of 69 peptides. Peptides arrived lyophilized and were re-suspended in DMSO (Sigma-Aldrich) at approximately 1-2 mg/mL. Stock peptides were kept frozen at –20ºC.

7.1.2 Human Peripheral Blood Mononuclear Cells

[0203] Community donor buffy coat products were purchased from the Stanford Blood Center, Palo Alto, CA. Buffy coat material was diluted 1:1 v:v with DPBS containing no calcium or magnesium. Diluted buffy coat material (25-35 mls) was underlayed in 50 ml conical centrifuge tubes (Sarsted or Costar) with 12.5 mls of FicollPaque-PLUS (GE Healthcare). The samples were centrifuged at 900 g for 30 minutes at room temperature. Peripheral blood mononuclear cells (PBMC) were collected from the interface. DPBS was added to bring the final volume to 50 mls and the cells were centrifuged at 350 g for 5 minutes. Pelleted cells were resuspended in DPBS and counted.

7.1.3 Dendritic cells

[0204] For isolation of dendritic cells, T75 culture flasks (Costar) were seeded with 10^8 freshly isolated PBMC in a total volume of 30 mls AIM V media (Invitrogen). Excess PBMC were frozen at –80ºC in 90% fetal calf serum (FCS), 10% DMSO at 5 x 10^7 cells/mL. T75 flasks were incubated at 37ºC in 5% CO_2 for 2 hours. Nonadherent cells were removed, and the adherent monolayer was washed with DPBS. To differentiate dendritic cells from monocytes, 30 mls of AIM V media containing 800 units/mL of GM-CSF (R and D Systems) and 500 units/mL IL-4 (R and D Systems) was added. Flasks were incubated for 5 days. On day 5 IL-1α (Endogen) and TNFα (Endogen) were added to 50 pg/mL and 0.2 ng/mL. Flasks were incubated two more days. On day 7, dendritic cells were collected by the addition of 3 mls of 100 mM EDTA containing 0.5 to 1.0 mg

Mitomycin C (Sigma-Aldrich) for a final concentration of 10 mM EDTA and 16.5 to 33 μg/mL Mitomycin C. Flasks were incubated an additional hour at 37°C and 5% CO_2. Dendritic cells were collected, and washed in AIM V media 2-3 times.

7.1.4 Cell culture

[0205] On day 7, previously frozen autologous PBMC were thawed quickly in a 37°C water bath. Cells were immediately diluted into DPBS or AIM V media and centrifuged at 350g for 5 minutes. $CD4^+$ cells were enriched by negative selection using magnetic beads (Easy-Sep $CD4^+$ kit, Stem Cell Technologies). Autologous $CD4^+$ T cells and dendritic cells were cocultured at 2 x 10^5 $CD4^+$ T cells per 2 x 10^4 dendritic cells per well in 96 well round bottomed plates (Costar 9077). Peptides were added at approximately 5 μg/mL. Control wells contained the DMSO (Sigma) vehicle alone at 0.25% v:v. Positive control wells contained DMSO at 0.25% and tetanus toxoid (List Biologicals or CalBioChem) at 1 μg/mL. Cultures were incubated for 5 days. On day 5, 0.25 μCi per well of tritiated thymidine (Amersham or GE Healthcare) was added. Cultures were harvested on day 6 to filtermats using a Packard Filtermate Cell harvester. Scintillation counting was performed using a Wallac MicroBeta 1450 scintillation counter (Perkin Elmer).

7.1.5 Data Analysis

[0206] Average background CPM values were calculated by averaging individual results from 6 to 12 replicates. The CPM values of the four positive control wells were averaged. Replicate or triplicate wells for each peptide were averaged. Stimulation index values for the positive control and the peptide wells were calculated by dividing the average experimental CPM values by the average control values. In order to be included in the dataset, a stimulation index of greater than 3.0 in the tetanus toxoid positive control wells was required. A response was noted for any peptide resulting in a stimulation index of 2.95 or greater. Peptides were tested using peripheral blood samples from a group of 99 donors. Responses to all peptides were compiled. For each peptide tested, the percentage of the donor set that responded with a stimulation index of 2.95 or greater was calculated. In addition, the average stimulation index for all donors was also calculated.

7.2 RESULTS

7.2.1 Identification Of CD4+ T Cell Epitopes In The Bevacizumab VH And VL Regions

[0207] $CD4^+$ T cell epitope peptides were identified by an analysis of the percent responses to the peptides within the set of 99 donors. The average percent response and standard deviation were calculated for all peptides tested describing the bevacizumab heavy chain and light chain V regions. A response rate greater than or equal to the average background response plus three standard deviations was considered a potential $CD4^+$ T cell epitope. For the bevacizumab light chain V region, 32 peptides were tested (Table 3) which resulted in an average background percent response of 2.1 ± 2.7%. Three standard deviations above background was determined to be 10.2%. One peptide at position 13 (Q40-T54) displayed this level of response in the bevacizumab light chain peptide dataset, with a response rate of 15.2% (Figure 2A). For the bevacizumab heavy chain V region, 37 peptides were tested (Table 4). The average background percent response was 2.8 ± 3.1%. Three standard deviations above background was 12.1%. One peptide within the bevacizumab heavy chain dataset, #18 (N52-R56), achieved a percent response of 16.2% (Figure 3A). A second peptide at position #30 in the heavy chain dataset achieved a response rate of 9.1%, and was considered an epitope due to an increased stimulation index (see below).

[0208] The average stimulation index was calculated for all peptides in the dataset. Light chain peptide 13 had a high average stimulation index of 1.82 ± 0.24 s.e.m. (Figure 2B). Heavy chain peptide #18 had an average stimulation index value of 2.16 ± 0.35 s.e.m. (Figure 3B). The peptide at position #30 returned an average stimulation index of 1.45 + 0.18 s.e.m. (Figure 3B) due to an elevated average stimulation index and an above average response rate. The peptide at position #30 was included when determining $CD4^+$ T cell epitope content of this antibody V region. All of these stimulation index values are significantly higher than the average stimulation index for all peptides in the two datasets (1.14 ± 0.07 for all 69 heavy chain and light chain peptides).

[0209] These data indicate that there are three CD4+ T cell epitope regions in the bevacizumab V regions (Table 5). In the VL region, an epitope is found at peptide position 13 that encompasses framework 2 and two amino acids from CDR2. The sequence contains a murine back-mutation (V46) inserted into the sequence during humanization. In Table 5, the CDR-derived amino acids are underlined. In the heavy chain, two epitope peptide regions were identified. The stronger epitope at position #18 encompasses all of CDR2. The second epitope peptide region contains both framework 3 and CDR3 amino acids.

7.2.2 Reduced Immunogenicity Variants of Bevacizumab Variant Antibodies

[0210] Bevacizumab was subjected to mutational analysis (see Example 2 below). Based on antigen-binding studies performed in conjunction with the mutational analysis, a set of candidate amino acid substitutions within the CDR-H2 and CDR-H3 region were identified that did not significantly reduce the affinity of the antibody to VEGF (Table 6). These amino acid substitutions were tested singly and in combination to identify variants of bevacizumab with reduced immunogenicity as compared to the wild type antibody.

8. EXAMPLE 2: IDENTIFICATION OF VARIANTS OF BEVACIZUMAB WITH INCREASED AFFINITY TO VEGF

[0211] The bevacizumab antibody was subjected to comprehensive mutational analysis to identify mutants that had increased affinity to VEGF as compared to bevacizumab. The increased affinity of candidate high affinity mutants to VEGF as compared to bevacizumab was analyzed by BIAcore to confirm their binding characteristics.

8.1 MATERIALS & METHODS

8.1.1 BIAcore

[0212] Fifteen variant bevacizumab VH region constructs were cloned along with the unmodified VL region into a human IgG_1-containing plasmid, expressed in 293T/17 cell lines by transient transfection, and antibodies purified by Protein A or Protein G affinity. The affinity of the antibodies for VEGF (R&D systems, Minneapolis, MN) was determined by using a BIAcore 2000 and 3000 surface plasmon resonance system (BIAcore, GE Healthcare, Piscataway, NJ). Polyclonal goat anti-human Fc antibody (Jackson Immunoresearch) was first immobilized to the biosensor surface using standard BIAcore amine coupling reagents (N-ethyl-N'-dimethylamino-propylcarbodiimide, EDC; N-hydroxysuccinimide, NHS; and ethanolamine HCl, pH 8.5), followed by the capture of anti-VEGF antibodies (bevacizumab and bevacizumab variants) on parallel surfaces at a low flow rate of 5 μL/min. RL was kept low to minimize avidity due to the dimeric nature of VEGF. No capture of the antibody was made on the reference surface to serve as a negative control. Subsequently, VEGF was injected to all flow cells at a flow rate of 50 μL/min for two minutes to monitor association followed by a 25-minute flow of HBS-P running buffer (10 mM HEPES, 150 mM sodium chloride, 0.005% P-20, pH 7.4) to monitor the dissociation phase. At each cycle, VEGF, in 6 different concentrations of VEGF ranging between 0 nM and 512nM and at four-fold increments, was injected over the surface. The surface was regenerated with 1.5% H_3PO_4 at a flow rate of 100 μL/min in two brief pulses at the end of each cycle. Binding data were fit to the 1:1 Langmuir model to extract binding constants from the BIAevaluate software. Double referencing was applied in each analysis to eliminate background responses from the reference surface and buffer only control. All the binding kinetics data were analyzed at least three separate determinations.

8.2 RESULTS

[0213] Results are displayed as absolute numbers and as fold improvement over wild-type. Almost all the variants listed have improved association (k_{on}) and dissociation (k_{off}) rates when compared to bevacizumab or wild-type (Table 7). The final affinity values for the variants were in the 0.1 nM range and reach as low as 0.08 nM for the variant corresponding to SEQ ID NO:82. These values contrast to bevacizumab which has a measured affinity in these experiments of 1.9 nM.

[0214] Tables 8 and 9 show additional heavy chain variants that preliminary binding studies show have a greater affinity to VEGF than bevacizumab (data not shown). Table 10 shows heavy chain variants that preliminary studies indicate have an affinity to VEGF similar to that of bevacizumab (data not shown). Table 11 shows light chain variants that that preliminary studies indicate have an affinity to VEGF similar to that of bevacizumab (data not shown).

9. EXAMPLE 3: SELECTION OF DEIMMUNIZED VARIANT PEPTIDES

[0215] Variant peptides corresponding to the immunogenic regions of bevacizumab (*see* Example 1) were generated (Tables 14–16). The variant peptides were selected on the basis of comprehensive mutational analysis described in Example 2, in which CDR modifications were identified that did not substantially reduce the binding affinity of bevacizumab to VEGF.

[0216] A total of 77 peptides were synthesized and tested based on the antigen-binding studies, including two syntheses of each of the parent 15-mer peptides. A total of 93 donors were tested with the parent and variant peptides utilizing the method described in Section 7.1 and the results are shown in Figures 4A–4C. In particular, Figures 4A-4C show CD4+ T cell responses to mutant bevacizumab epitope peptides. Average responses to the unmodified parent epitope sequences are indicated with open marks. Large circles indicate selected peptides referred to in Table 17 (see below). Figure 4A shows VH CDR2 peptides; Figure 4B shows VH CDR3 peptides; and Figure 4C shows VL CDR2 peptides. Immunogenicity data for selected peptides are shown in Table 17.

[0217] For the heavy chain variable region CDR2 peptides, the average percent response to the parent peptides in this study was 5.38% and 6.45%. Three mutant peptides demonstrated a reduced overall response rate and average stimulation index as compared to the parent peptides.

[0218] The parent peptide response rates for the heavy chain variable region CDR3 epitope peptides in this study were 7.53% and 6.45%. A single mutant peptide sequence was found that demonstrated reduced overall responses as compared to the parent peptide.

[0219] Finally, the light chain variable region CDR2 peptide parent response rates in this study were 25.8% and 15%. One mutant peptide was identified that demonstrated a reduced overall immunogenicity as compared to the parent peptide.

[0220] To demonstrate that the deimmunizing mutations maintained affinity to VEGF as compared to bevacizumab, flow cytometry was used to compare the binding properties of variant antibodies incorporating mutations in the modified epitope peptides (either as single or double amino-acid modifications and bevacizumab). Several deimmunizing mutations had comparable or increased affinity to VEGF as compared to bevacizumab.

[0221] In one study, transiently transfected 293c18 cells expressing surface-bound forms of the bevacizumab variants were stained with Alexa647-conjugated rHuVEGF (Invitrogen Cat #PHG0143) at 3 nM and goat-anti-human-kappa-RPE (Southern Biotech Cat#2063-09) at a 1:400 dilution. Data were gathered by way of flow cytometry using a

DakoCytomation CyAn ADP flow cytometer and was analyzed using Treestar's FloJo analysis program. The mean fluorescence intensities (MFI) measured in this work are set forth in Table 18.

[0222] In another study, the EC_{50} of bevacizumab and variant antibody binding to VEGF was measured. Antibody titration plots were generated using bevacizumab and its variants with Alexas647-conjugated rHu VEGF as described above, with the VEGF serially diluted two-fold from 5 μM to 0.01 μM. The EC_{50} values are shown in Table 19.

10. SPECIFIC EMBODIMENTS, CITATION OF REFERENCES

[0223] All publications, patents, patent applications and other documents cited in this application are hereby incorporated by reference in their entireties for all purposes to the same extent as if each individual publication, patent, patent application or other document were individually indicated to be incorporated by reference for all purposes. While various specific embodiments have been illustrated and described, it will be appreciated that various changes can be made without departing from the spirit and scope of the invention(s).

WHAT IS CLAIMED IS:

1. An anti-VEGF antibody or an anti-VEGF binding fragment of an antibody which comprises CDRs having amino acid sequences corresponding to SEQ ID NO:3 (CDR-H1), SEQ ID NO:4 (CDR-H2), SEQ ID NO:5 (CDR-H3), SEQ ID NO:6 (CDR-L1), SEQ ID NO:7 (CDR-L2) and SEQ ID NO:8 (CDR-L3), wherein CDR-H2 includes the substitution K64S, and optionally one or more additional mutations or combinations of mutations selected from one or more of Tables 6, 7, 8, 9, 10, 11, 12-1 to 12-9, or 13-16, wherein the six CDRs altogether have up to 17 amino acid substitutions as compared to CDR sequences of the antibody bevacizumab or the antibody ranibizumab.

2. An anti-VEGF antibody or an anti-VEGF binding fragment of an antibody which comprises CDRs having amino acid sequences corresponding to SEQ ID NO:3 (CDR-H1), SEQ ID NO:4 (CDR-H2), SEQ ID NO:5 (CDR-H3), SEQ ID NO:6 (CDR-L1), SEQ ID NO:7 (CDR-L2) and SEQ ID NO:8 (CDR-L3), wherein CDR-H2 includes the substitution K64Q, and optionally one or more additional mutations or combinations of mutations selected from one or more of Tables 6, 7, 8, 9, 10, 11, 12-1 to 12-9, or 13-16, wherein the six CDRs altogether have up to 17 amino acid substitutions as compared to CDR sequences of the antibody bevacizumab or the antibody ranibizumab.

3. An anti-VEGF antibody or an anti-VEGF binding fragment of an antibody which comprises CDRs having amino acid sequences corresponding to SEQ ID NO:3 (CDR-H1), SEQ ID NO:4 (CDR-H2), SEQ ID NO:5 (CDR-H3), SEQ ID NO:6 (CDR-L1), SEQ ID NO:7 (CDR-L2) and SEQ ID NO:8 (CDR-L3), wherein CDR-H2 includes the substitutions Y53F and K64Q, and optionally one or more additional mutations or combinations of mutations selected from one or more of Tables 6, 7, 8, 9, 10, 11, 12-1 to 12-9, or 13-16, wherein the six CDRs altogether have up to 17 amino acid substitutions as compared to CDR sequences of the antibody bevacizumab or the antibody ranibizumab.

4. An anti-VEGF antibody or an anti-VEGF binding fragment of an antibody which comprises CDRs having amino acid sequences corresponding to SEQ ID NO:3

(CDR-H1), SEQ ID NO:4 (CDR-H2), SEQ ID NO:5 (CDR-H3), SEQ ID NO:6 (CDR-L1), SEQ ID NO:7 (CDR-L2) and SEQ ID NO:8 (CDR-L3), wherein CDR-H3 includes the substitutions H97E and Y98F, and optionally one or more additional mutations or combinations of mutations selected from one or more of Tables 6, 7, 8, 9, 10, 11, 12-1 to 12-9, or 13-16, wherein the six CDRs altogether have up to 17 amino acid substitutions as compared to CDR sequences of the antibody bevacizumab or the antibody ranibizumab.

5. An anti-VEGF antibody or an anti-VEGF binding fragment of an antibody which comprises CDRs having amino acid sequences corresponding to SEQ ID NO:3 (CDR-H1), SEQ ID NO:4 (CDR-H2), SEQ ID NO:5 (CDR-H3), SEQ ID NO:6 (CDR-L1), SEQ ID NO:7 (CDR-L2) and SEQ ID NO:8 (CDR-L3), wherein CDR-L2 includes the substitution T51A and optionally one or more additional mutations or combinations of mutations selected from one or more of Tables 6, 7, 8, 9, 10, 11, 12-1 to 12-9, or 13-16, wherein the six CDRs altogether have up to 17 amino acid substitutions as compared to CDR sequences of the antibody bevacizumab or the antibody ranibizumab, and wherein CDR-L2 in said VEGF antibody does not consist of a CDR-L2 sequence set forth in Table 13.

6. An anti-VEGF antibody or an anti-VEGF binding fragment of an antibody which comprises six complementarity determining regions ("CDRs") having amino acid sequences corresponding to SEQ ID NO:3 (CDR-H1), SEQ ID NO:4 (CDR-H2), SEQ ID NO:5 (CDR-H3), SEQ ID NO:6 (CDR-L1), SEQ ID NO:7 (CDR-L2) and SEQ ID NO:8 (CDR-L3), wherein said CDR-H1 includes the substitution N31F, and optionally one or more additional mutations or combinations of mutations selected from one or more of Tables 6, 7, 8, 9, 10, 11, 12-1 to 12-9 and 13, wherein the six CDRs altogether have up to 17 amino acid substitutions as compared to CDR sequences of the antibody bevacizumab or the antibody ranibizumab, and wherein CDR-H1 in said VEGF antibody does not consist of a CDR-H1 sequence set forth in Tables 12-1 to 12-9.

7. An anti-VEGF antibody or an anti-VEGF binding fragment of an antibody which comprises six CDRs having amino acid sequences corresponding to SEQ ID NO:3

(CDR-H1), SEQ ID NO:4 (CDR-H2), SEQ ID NO:5 (CDR-H3), SEQ ID NO:6 (CDR-L1), SEQ ID NO:7 (CDR-L2) and SEQ ID NO:8 (CDR-L3), wherein said CDR-H3 includes the substitution H97E, and optionally one or more additional mutations or combinations of mutations selected from one or more of Tables 6, 7, 8, 9, 10, 11, 12-1 to 12-9 and 13, wherein the six CDRs altogether have up to 17 amino acid substitutions as compared to CDR sequences of the antibody bevacizumab or the antibody ranibizumab, and wherein CDR-H3 in said VEGF antibody does not consist of a CDR-H3 sequence set forth in Tables 12-1 to 12-9.

8. An anti-VEGF antibody or an anti-VEGF binding fragment of an antibody which comprises six CDRs having amino acid sequences corresponding to SEQ ID NO:3 (CDR-H1), SEQ ID NO:4 (CDR-H2), SEQ ID NO:5 (CDR-H3), SEQ ID NO:6 (CDR-L1), SEQ ID NO:7 (CDR-L2) and SEQ ID NO:8 (CDR-L3), wherein said CDR-H3 includes the substitution H97D, and optionally one or more additional mutations or combinations of mutations selected from one or more of Tables 6, 7, 8, 9, 10, 11, 12-1 to 12-9 and 13, wherein the six CDRs altogether have up to 17 amino acid substitutions as compared to CDR sequences of the antibody bevacizumab or the antibody ranibizumab, and wherein CDR-H3 in said VEGF antibody does not consist of a CDR-H3 sequence set forth in Tables 12-1 to 12-9.

9. An anti-VEGF antibody or an anti-VEGF binding fragment of an antibody which comprises six CDRs having amino acid sequences corresponding to SEQ ID NO:3 (CDR-H1), SEQ ID NO:4 (CDR-H2), SEQ ID NO:5 (CDR-H3), SEQ ID NO:6 (CDR-L1), SEQ ID NO:7 (CDR-L2) and SEQ ID NO:8 (CDR-L3), wherein said CDR-H3 includes the substitution H97P, and optionally one or more additional mutations or combinations of mutations selected from one or more of Tables 6, 7, 8, 9, 10, 11, 12-1 to 12-9 and 13, wherein the six CDRs altogether have up to 17 amino acid substitutions as compared to CDR sequences of the antibody bevacizumab or the antibody ranibizumab, and wherein CDR-H3 in said VEGF antibody does not consist of a CDR-H3 sequence set forth in Tables 12-1 to 12-9.

10. An anti-VEGF antibody or an anti-VEGF binding fragment of an antibody which comprises six CDRs having amino acid sequences corresponding to SEQ ID NO:3

(CDR-H1), SEQ ID NO:4 (CDR-H2), SEQ ID NO:5 (CDR-H3), SEQ ID NO:6 (CDR-L1), SEQ ID NO:7 (CDR-L2) and SEQ ID NO:8 (CDR-L3), wherein said CDR-H3 includes the substitution Y99E, and optionally one or more additional mutations or combinations of mutations selected from one or more of Tables 6, 7, 8, 9, 10, 11, 12-1 to 12-9 and 13, wherein the six CDRs altogether have up to 17 amino acid substitutions as compared to CDR sequences of the antibody bevacizumab or the antibody ranibizumab, and wherein CDR-H3 in said VEGF antibody does not consist of a CDR-H3 sequence set forth in Tables 12-1 to 12-9.

11. An anti-VEGF antibody or an anti-VEGF binding fragment of an antibody which comprises six CDRs having amino acid sequences corresponding to SEQ ID NO:3 (CDR-H1), SEQ ID NO:4 (CDR-H2), SEQ ID NO:5 (CDR-H3), SEQ ID NO:6 (CDR-L1), SEQ ID NO:7 (CDR-L2) and SEQ ID NO:8 (CDR-L3), wherein said CDR-H3 includes the substitution Y99D, and optionally one or more additional mutations or combinations of mutations selected from one or more of Tables 6, 7, 8, 9, 10, 11, 12-1 to 12-9 and 13, wherein the six CDRs altogether have up to 17 amino acid substitutions as compared to CDR sequences of the antibody bevacizumab or the antibody ranibizumab, and wherein CDR-H3 in said VEGF antibody does not consist of a CDR-H3 sequence set forth in Tables 12-1 to 12-9.

12. An anti-VEGF antibody or an anti-VEGF binding fragment of an antibody which comprises six CDRs having amino acid sequences corresponding to SEQ ID NO:3 (CDR-H1), SEQ ID NO:4 (CDR-H2), SEQ ID NO:5 (CDR-H3), SEQ ID NO:6 (CDR-L1), SEQ ID NO:7 (CDR-L2) and SEQ ID NO:8 (CDR-L3), wherein said CDR-H3 includes the substitution S100aG, and optionally one or more additional mutations or combinations of mutations selected from one or more of Tables 6, 7, 8, 9, 10, 11, 12-1 to 12-9 and 13, wherein the six CDRs altogether have up to 17 amino acid substitutions as compared to CDR sequences of the antibody bevacizumab or the antibody ranibizumab, and wherein CDR-H3 in said VEGF antibody does not consist of a CDR-H3 sequence set forth in Tables 12-1 to 12-9.

13. An anti-VEGF antibody or an anti-VEGF binding fragment of an antibody which comprises CDRs having amino acid sequences corresponding to SEQ ID NO:3

(CDR-H1), SEQ ID NO:4 (CDR-H2), SEQ ID NO:5 (CDR-H3), SEQ ID NO:6 (CDR-L1), SEQ ID NO:7 (CDR-L2) and SEQ ID NO:8 (CDR-L3), wherein said CDR-H3 includes the substitution S100aG, and optionally one or more additional mutations or combinations of mutations selected from one or more of Tables 6, 7, 8, 9, 10, 11, 12-1 to 12-9 and 13, wherein the six CDRs altogether have up to 17 amino acid substitutions as compared to CDR sequences of the antibody bevacizumab or the antibody ranibizumab, and wherein position 3 in CDR-H3 of said VEGF antibody is not tyrosine.

14. An anti-VEGF antibody or an anti-VEGF binding fragment of an antibody which comprises CDRs having amino acid sequences corresponding to SEQ ID NO:3 (CDR-H1), SEQ ID NO:4 (CDR-H2), SEQ ID NO:5 (CDR-H3), SEQ ID NO:6 (CDR-L1), SEQ ID NO:7 (CDR-L2) and SEQ ID NO:8 (CDR-L3), wherein CDR-H1 includes at least one substitution selected from T28P, N31F, N31G and N31M, and optionally one or more additional mutations or combinations of mutations selected from one or more of Tables 6, 7, 8, 9, 10, 11, 12-1 to 12-9 and 13, wherein the six CDRs altogether have up to 17 amino acid substitutions as compared to CDR sequences of the antibody bevacizumab or the antibody ranibizumab, and wherein CDR-H1 in said VEGF antibody does not consist of a CDR-H1 sequence set forth in Tables 12-1 to 12-9.

15. An anti-VEGF antibody or an anti-VEGF binding fragment of an antibody which comprises CDRs having amino acid sequences corresponding to SEQ ID NO:3 (CDR-H1), SEQ ID NO:4 (CDR-H2), SEQ ID NO:5 (CDR-H3), SEQ ID NO:6 (CDR-L1), SEQ ID NO:7 (CDR-L2) and SEQ ID NO:8 (CDR-L3), wherein CDR-H3 includes at least one substitution selected from H97A, H97Q, H97S, H97T, S100aD, S100aE, and S100aV, and optionally one or more additional mutations or combinations of mutations selected from one or more of Tables 6, 7, 8, 9, 10, 11, 12-1 to 12-9 and 13, wherein the six CDRs altogether have up to 17 amino acid substitutions as compared to CDR sequences of the antibody bevacizumab or the antibody ranibizumab, and wherein CDR-H3 in said VEGF antibody does not consist of a CDR-H3 sequence set forth in Tables 12-1 to 12-9.

16. An anti-VEGF antibody or an anti-VEGF binding fragment of an antibody which comprises CDRs having amino acid sequences corresponding to SEQ ID NO:3 (CDR-H1), SEQ ID NO:4 (CDR-H2), SEQ ID NO:5 (CDR-H3), SEQ ID NO:6 (CDR-L1), SEQ ID NO:7 (CDR-L2) and SEQ ID NO:8 (CDR-L3), wherein CDR-H1 includes the subsitution T30W, T30R or T30Q, and optionally one or more additional mutations or combinations of mutations selected from one or more of Tables 6, 7, 8, 9, 10, 11, 12-1 to 12-9 and 13, wherein the six CDRs altogether have up to 17 amino acid substitutions as compared to CDR sequences of the antibody bevacizumab or the antibody ranibizumab, and wherein CDR-H1 in said VEGF antibody does not consist of a CDR-H1 sequence set forth in Tables 12-1 to 12-9.

17. An anti-VEGF antibody or an anti-VEGF binding fragment of an antibody which comprises CDRs having amino acid sequences corresponding to SEQ ID NO:3 (CDR-H1), SEQ ID NO:4 (CDR-H2), SEQ ID NO:5 (CDR-H3), SEQ ID NO:6 (CDR-L1), SEQ ID NO:7 (CDR-L2) and SEQ ID NO:8 (CDR-L3), wherein CDR-H2 includes at least one substitution selected from Y53F, T58F, A61G, A61K, A61R, A61H, A61Y, K64G, K64E, R65L, R65T, R65A, R65E, and R65D, and optionally one or more additional mutations or combinations of mutations selected from one or more of Tables 6, 7, 8, 9, 10, 11, 12-1 to 12-9 and 13, wherein the six CDRs altogether have up to 17 amino acid substitutions as compared to CDR sequences of the antibody bevacizumab or the antibody ranibizumab, and wherein CDR-H2 in said VEGF antibody does not consist of a CDR-H2 sequence set forth in Tables 12-1 to 12-9.

18. An anti-VEGF antibody or an anti-VEGF binding fragment of an antibody which comprises CDRs having amino acid sequences corresponding to SEQ ID NO:3 (CDR-H1), SEQ ID NO:4 (CDR-H2), SEQ ID NO:5 (CDR-H3), SEQ ID NO:6 (CDR-L1), SEQ ID NO:7 (CDR-L2) and SEQ ID NO:8 (CDR-L3), wherein CDR-H3 includes at least one substitution selected from Y98F and Y100eF, and optionally one or more additional mutations or combinations of mutations selected from one or more of Tables 6, 7, 8, 9, 10, 11, 12-1 to 12-9 and 13, wherein the six CDRs altogether have up to 17 amino acid substitutions as compared to CDR

sequences of the antibody bevacizumab or the antibody ranibizumab, and wherein CDR-H3 in said VEGF antibody does not consist of a CDR-H3 sequence set forth in Tables 12-1 to 12-9.

19. An anti-VEGF antibody or an anti-VEGF binding fragment of an antibody which comprises CDRs having amino acid sequences corresponding SEQ ID NO:3 (CDR-H1), SEQ ID NO:4 (CDR-H2), SEQ ID NO:5 (CDR-H3), SEQ ID NO:6 (CDR-L1), SEQ ID NO:7 (CDR-L2) and SEQ ID NO:8 (CDR-L3), wherein the heavy chain CDRs include a combination of substitutions selected from:
   (a) N31F in CDR-H1, H97D in CDR-H3, Y99D in CDR-H3, and S100aG in CDR-H3;
   (b) N31F in CDR-H1, H97P in CDR-H3, Y99D in CDR-H3, and S100aG in CDR-H3;
   (c) N31F in CDR-H1, H97P in CDR-H3, and Y99E in CDR-H3;
   (d) N31F in CDR-H1, H97E in CDR-H3, and Y99E in CDR-H3;
   (e) N31F in CDR-H1, H97D in CDR-H3, and Y99E in CDR-H3;
   (f) N31F in CDR-H1, H97E in CDR-H3, Y99D in CDR-H3, and S100aG in CDR-H3;
   (g) N31F in CDR-H1, Y99D in CDR-H3, and S100aG in CDR-H3;
   (h) N31F in CDR-H1, H97P in CDR-H3, and Y99D in CDR-H3;
   (i) N31F in CDR-H1, H97D in CDR-H3, and S100aG in CDR-H3;
   (j) N31F in CDR-H1 and S100aG in CDR-H3;
   (k) N31F in CDR-H1, H97P in CDR-H3, and S100aG in CDR-H3;

   wherein said antibody or binding fragment optionally comprises one or more additional mutations or combinations of mutations selected from one or more of Tables 6, 7, 8, 9, 10, 11, 12-1 to 12-9 and 13, wherein the six CDRs altogether have up to 17 amino acid substitutions as compared to CDR sequences of the antibody bevacizumab or the antibody ranibizumab.

20. The anti-VEGF antibody or anti-VEGF binding fragment of claim 19, in which, other than the combination of substitutions selected from (a) to (k), the heavy chain CDRs do not comprise additional mutations as compared to the heavy chain

CDR sequences of the antibody bevacizumab or as compared to the heavy chain CDR sequences of the antibody ranibizumab.

21. The anti-VEGF antibody or anti-VEGF binding fragment of any one of claims 1 to 19, wherein the six CDRs altogether have up to 16, up to 15, up to 14, up to 13, up to 12, up to 11, up to 10, up to 9, up to 8, up to 7, up to 6, up to 5, or up to 4 amino acid substitutions as compared to CDR sequences of the antibody bevacizumab or the antibody ranibizumab.

22. The anti-VEGF antibody or anti-VEGF binding fragment of any one of claims 1 to 21, wherein any individual CDR has no more than three amino acid substitutions as compared to the corresponding CDR sequence of the antibody bevacizumab or the antibody ranibizumab.

23. The anti-VEGF antibody or anti-VEGF binding fragment of anti-VEGF antibody or anti-VEGF binding fragment of any one of claims 1 to 21, wherein any individual CDR has no more than two amino acid substitutions as compared to the corresponding CDR sequence of the antibody bevacizumab or the antibody ranibizumab.

24. The anti-VEGF antibody or anti-VEGF binding fragment of any one of claims 1 to 23 which is a monoclonal antibody or anti-VEGF binding fragment of a monoclonal antibody, respectively.

25. The anti-VEGF antibody or an anti-VEGF binding fragment of any one of claims 1 to 24 which is a human or humanized antibody, or anti-VEGF binding fragment of a humanized or humanized antibody, respectively.

26. The anti-VEGF antibody or an anti-VEGF binding fragment of any one of claims 1 to 25 which is an IgG.

27. The anti-VEGF antibody or an anti-VEGF binding fragment of claim 26 which is an IgG_1.

28. The anti-VEGF antibody or an anti-VEGF binding fragment of claim 26 which is an IgG_2.

29. The anti-VEGF antibody or an anti-VEGF binding fragment of any one of claims 1 to 28 which includes one or more mutations in the Fc region that increase ADCC activity.

30. The anti-VEGF antibody or an anti-VEGF binding fragment of any one of claims 1 to 29 which is non-fucosylated.

31. The anti-VEGF antibody or an anti-VEGF binding fragment of any one of claims 1 to 30 which includes one or more mutations in the Fc region that increase binding to FcγR.

32. The anti-VEGF antibody or an anti-VEGF binding fragment of any one of claims 1 to 30 which includes one or more mutations in the Fc region that increase binding to FcRn.

33. The anti-VEGF antibody or an anti-VEGF binding fragment of any one of claims 1 to 27 which includes one or more mutations in the Fc region that decrease ADCC activity.

34. The anti-VEGF antibody or an anti-VEGF binding fragment of any one of claims 1 to 34 which has, other than said one or more mutations, a V_H sequence corresponding to SEQ ID NO:1 and a V_L sequence corresponding to SEQ ID NO:2.

35. The anti-VEGF antibody or an anti-VEGF binding fragment of any one of claims 1 to 34 which has, other than said one or more mutations, a heavy chain sequence corresponding to SEQ ID NO:9 and a light chain sequence corresponding to SEQ ID NO:10.

36. The anti-VEGF antibody or anti-VEGF binding fragment of any one of claims 1 to 34 which has an affinity that is 1.5- to 50-fold greater than the affinity of an

antibody having a V_H sequence corresponding to SEQ ID NO:1 and a V_L sequence corresponding to SEQ ID NO:2.

37. The anti-VEGF antibody or anti-VEGF binding fragment of any one of claims 1 to 34 which has an affinity that is 1.5- to 50-fold greater than the affinity of an antibody having a heavy chain sequence corresponding to SEQ ID NO:9 and a light chain sequence corresponding to SEQ ID NO:10.

38. The anti-VEGF antibody or anti-VEGF binding fragment of claim 36 which has a 2- to 30-fold greater than the affinity of an antibody having a V_H sequence corresponding to SEQ ID NO:1 and a V_L sequence corresponding to SEQ ID NO:2.

39. The anti-VEGF antibody or anti-VEGF binding fragment of claim 37 which has a 2- to 30-fold greater than the affinity of an antibody having a heavy chain sequence corresponding to SEQ ID NO:9 and a light chain sequence corresponding to SEQ ID NO:10

40. The anti-VEGF antibody or anti-VEGF binding fragment of any one of claims 36 to 39, wherein the affinity is a measure of K_D as analyzed by a biosensor.

41. The anti-VEGF antibody or anti-VEGF binding fragment of any one of claims 1 to 40 which is purified.

42. The anti-VEGF antibody or anti-VEGF binding fragment of claim 41 which is purified to at least 85%, at least 90%, at least 95% or at least 98% homogeneity.

43. An anti-VEGF antibody or an anti-VEGF binding fragment of an antibody which comprises six complementarity determining regions ("CDRs") having amino acid sequences corresponding to SEQ ID NO:3 (CDR-H1), SEQ ID NO:4 (CDR-H2), SEQ ID NO:5 (CDR-H3), SEQ ID NO:6 (CDR-L1), SEQ ID NO:7 (CDR-L2) and SEQ ID NO:8 (CDR-L3), wherein the heavy chain includes at least one substitution selected from A61F in CDR-H2, A61E in CDR-H2, A61D in CDR-H2, D62L in CDR-H2, D62G in CDR-H2, D62Q in CDR-H2, D62T in CDR-H2, D62K in CDR-H2, D62R in CDR-H2, D62E in CDR-H2, D62H in CDR-H2,

K64S in CDR-H2, K64V in CDR-H2, K64Q in CDR-H2, R65V in CDR-H2, R65F in CDR-H2, R65H in CDR-H2, R65N in CDR-H2, R65S in CDR-H2, R65Q in CDR-H2, R65K in CDR-H2, R65I in CDR-H2, and Y98H in CDR-H3, and optionally one or more additional mutations or combinations of mutations selected from one or more of Tables 7, 8, 9, 10, 11, 12-1 to 12-9 and 13, wherein the six CDRs altogether have up to 17 amino acid substitutions as compared to CDR sequences of the antibody bevacizumab or the antibody ranibizumab.

44. The anti-VEGF antibody or an anti-VEGF binding fragment of claim 43 wherein wherein the six CDRs altogether have up to 16, up to 15, up to 14, up to 13, up to 12, up to 11, up to 10, up to 9, up to 8, up to 7, up to 6, up to 5, or up to 4 amino acid substitutions as compared to CDR sequences of the antibody bevacizumab or the antibody ranibizumab.

45. The anti-VEGF antibody or an anti-VEGF binding fragment of claim 43 or claim 44, wherein any individual CDR has no more than three amino acid substitutions as compared to the corresponding CDR sequence of the antibody bevacizumab.

46. The anti-VEGF antibody or an anti-VEGF binding fragment of claim 43 or claim 44, wherein any individual CDR has no more than two amino acid substitutions as compared to the corresponding CDR sequence of the antibody bevacizumab.

47. The anti-VEGF antibody or anti-VEGF binding fragment of any one of claims 43 to 46 which is a monoclonal antibody or an anti-VEGF binding fragment of a monoclonal antibody, respectively.

48. The anti-VEGF antibody or an anti-VEGF binding fragment of any one of claims 43 to 47 which is a human or humanized antibody, or an anti-VEGF binding fragment of a humanized or humanized antibody, respectively.

49. The anti-VEGF antibody or an anti-VEGF binding fragment of any one of claims 43 to 48 which is an IgG.

50. The anti-VEGF antibody or an anti-VEGF binding fragment of claim 49 which is an IgG_1.

51. The anti-VEGF antibody or an anti-VEGF binding fragment of claim 49 which is an IgG_2.

52. The anti-VEGF antibody or an anti-VEGF binding fragment of any one of claims 43 to 51 which includes one or mutations in the Fc region that increase ADCC activity.

53. The anti-VEGF antibody or an anti-VEGF binding fragment of any one of claims 43 to 52 which is non-fucosylated.

54. The anti-VEGF antibody or an anti-VEGF binding fragment of any one of claims 43 to 53 which includes one or more mutations in the Fc region that increase binding to FcγR.

55. The anti-VEGF antibody or an anti-VEGF binding fragment of any one of claims 43 to 53 which includes one or more mutations in the Fc region that increase binding to FcRn.

56. The anti-VEGF antibody or an anti-VEGF binding fragment of any one of claims 43 to 50 which includes one or mutations in the Fc region that decrease ADCC activity.

57. The anti-VEGF antibody or an anti-VEGF binding fragment of any one of claims 43 to 56 which has, other than said one or more mutations, a V_H sequence corresponding to SEQ ID NO:1 and a V_L sequence corresponding to SEQ ID NO:2.

58. The anti-VEGF antibody or anti-VEGF binding fragment of any one of claims 43 to 57 which is purified.

59. The anti-VEGF antibody or anti-VEGF binding fragment of claim 58 which is purified to at least 85%, at least 90%, at least 95% or at least 98% homogeneity.

60. An antibody-drug conjugate comprising an anti-VEGF antibody or anti-VEGF binding fragment according to any one of claims 1 to 59.

61. A pharmaceutical composition comprising an anti-VEGF antibody or anti-VEGF binding fragment according to any one of claims 1 to 59 or an antibody-drug conjugate according to claim 60, and a pharmaceutically acceptable carrier.

62. A nucleic acid comprising a nucleotide sequence encoding anti-VEGF antibody or anti-VEGF binding fragment of any one of claims 1 to 36 and 43 to 57.

63. A vector comprising the nucleic acid of claim 62.

64. A prokaryotic host cell transformed with a vector according to claim 63.

65. A eukaryotic host cell transformed with a vector according to claim 63.

66. A eukaryotic host cell engineered to express the nucleotide sequence of claim 62.

67. The eukaryotic host cell of claim 66 which is a mammalian host cell.

68. A method of producing anti-VEGF antibody or anti-VEGF binding fragment, comprising: (a) culturing the eukaryotic host cell of claim 66 or claim 67 and (b) recovering the anti-VEGF antibody or anti-VEGF binding fragment antibody.

69. A method of treating cancer, comprising administering to a human patient in need thereof a therapeutically effective amount of anti-VEGF antibody or anti-VEGF binding fragment according to any one of claims 1 to 59, an antibody-drug conjugate according to claim 60, or a pharmaceutical composition according to claim 61.

70. The method of claim 69, wherein the cancer is metastatic carcinoma of the colon, metastatic carcinoma of the rectum, non-squamous non-small cell lung cancer, or metastatic HER2-negative breast cancer.

71. The method of claim 70, wherein the cancer is non-squamous non-small cell lung cancer which is unresectable, locally advanced, recurrent or metastatic.

72. A method of treating age-related macular degeneration, comprising administering to a human patient in need thereof a therapeutically effective amount of anti-

VEGF antibody or anti-VEGF binding fragment according to any one of claims 1 to 59, an antibody-drug conjugate according to claim 60, or a pharmaceutical composition according to claim 61.

73. A method of treating an immune disorder, comprising administering to a human patient in need thereof a therapeutically effective amount of anti-VEGF antibody or anti-VEGF binding fragment according to any one of claims 1 to 59, an antibody-drug conjugate according to claim 60, or a pharmaceutical composition according to claim 61.

74. The method of claim 73, wherein the immune disorder is rheumatoid arthritis or Grave's disease.